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BLOOD AND SYMPTOMATIC CHANGES FOLLOWING THE INTRAVENOUS ADMINISTRATION OF A VARIETY OF AGENTS AND SOLUTIONS *

P. J. HANZLIK, M.D.
F. DE EDS, Ph.D.
AND
M. L. TAINTER, M.D.
SAN FRANCISCO

The object of this study was to observe the changes in composition and appearance of the blood occurring immediately after the intravenous injection of a variety of agents. These included some that cause anaphylactoid reactions in animals and some that are exploited for therapeutic purposes in human subjects. In this way, additional information regarding the effects of intravenous injections could be secured, and the hypothesis of hemoclasis or colloidoclasis, that is to say, disturbances in physical and chemical mechanisms of the blood and tissues, as the explanation of the changes and reactions could be tested. The analysis of blood was limited to the more important constituents and changes that might accompany such states as collapse, anoxemia, asphyxia, intravascular coagulation and thrombosis, which occur in varying degrees during the acute reactions from many agents injected intravenously. These have been described previously by several investigators. The literature of the subject has been reviewed in the publications of Hanzlik and Karsner [1] and recently brought up to date by one of us.[2] It will therefore be omitted in this paper, except for a few of the more important and recent papers related to our work.

Numerous details recorded in these publications agree with the views that the anaphylactoid reactions rest on a physical-chemical basis, and that intravenous medication in general is dangerous. This holds even

* From the Department of Pharmacology, Stanford University School of Medicine.

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1. Hanzlik, P. J., and Karsner, H. T.: J. Pharm. & Exper. Therap. **14**:379, 425 (Jan.); 449, 463, 479 (Feb.) 1920; ibid. **23**:173, 237, 243 (April) 1924.

2. Hanzlik, P. J.: Basis of Allergic Phenomena, J. A. M. A. **82**:2001 (June 21) 1924.

for agents which appear to be relatively inert but which, nevertheless, act as foreign bodies in the blood stream and produce contact phenomena that determine the physical and chemical changes and mediate functional changes in the cells variously recognizable as symptoms and reactions. The results of the present study corroborate the views expressed by several investigators. In fact, they constitute a measure of proof of physical and chemical disturbances and confirm the limited reports on a few agents previously studied by others.

METHODS

In their studies on anaphylactoid phenomena, Hanzlik and Karsner [1] used guinea-pigs and some cats. In the present study, dogs were used throughout, because the extensive analyses that were made necessitated larger quantities of blood than can be obtained from smaller animals. Certain of the agents that were used, for example, histamin, are known to give different symptomatic responses in dogs than in guinea-pigs; but others, namely, arsphenamin, acacia, agar, copper sulphate and kaolin, were found by us to give practically identical responses in the two species. In work going on in this laboratory with pigeons, the symptomatic responses are similar to those of guinea-pigs, and the chemical and physical changes in the blood are similar to those of dogs. In the agglutination studies of Karsner and Hanzlik,[1] practically identical responses were obtained with cat, human and guinea-pig bloods. Hence, the general results in different species appear to agree fairly well and further justify the use of dogs in the present connection for obtaining an idea of the blood and tissue changes after the intravenous injection of agents causing anaphylactoid reactions.

Both morphinized and unmorphinized dogs with and without artificial respiration were used. When morphin was used, the dosage ranged from 0.25 to 0.5 c.c. of 4 per cent. solution per kilogram subcutaneously. Light ether anesthesia was administered to all animals for the minor operative procedures that were carried out, namely, exposure and connection of the carotid artery for blood pressure record, insertion of a T-tracheal cannula and exposure of, and insertion of cannulas into, the saphenous vein and femoral artery. Then the ether anesthesia was discontinued during the remainder of the experiment. Kymographic records were made of blood pressure from a mercury manometer and of respiration from a tambour connected with a Woulff bottle, which, in turn, was joined to the tracheal cannula, the side arms of which was open so as to avoid asphyxia. Changes in temperature were recorded from a rectal thermometer. Careful records of symptoms and pulse and respiratory rates also were made.

All the agents and solutions were previously warmed and injected slowly into the saphenous vein from a buret. As a rule, the dosage was small and corresponded to that used for therapeutic purposes in human subjects. The doses of all agents used are recorded in the tables. In the majority of dogs more than one agent or solution was injected. This was done for economical reasons and also to observe changes under as many different conditions as possible. In many instances, the composition of the blood was changed by the first injection, so that the changes from subsequent injections of the same or different agents were additive. However, by reversing the order of injection of the agents, it was possible to secure first effects from all agents and also their effects under modified conditions of the preceding injections. In many instances, the changes produced were temporary and subsequent injections gave practically uncomplicated results. In any case, all conditions pertaining to such functions as respiration, blood pressure and temperature, and the composition of the blood were noted. In this way, we were able to secure economically numerous results under uncomplicated and complicated conditions, all of which served the purpose of the study. Certain complications, for example, circulatory collapse and

shock, which supervened on the first injection, served the purpose in that such conditions constitute indications for treatment by certain solutions (acacia and hypertonic sugar and salt solutions), according to their advocates. And it is in such conditions, rather than in health, that the blood, tissue and symptomatic changes are of practical interest.

Blood for the analysis was almost invariably drawn from the femoral artery through a long, bent glass cannula, the distal or free end of which was under from 10 to 15 c.c. of clean liquid petrolatum in a large test tube containing a little dry oxalate. In this way loss of carbon dioxid was reduced to the minimum. Between 15 and 17 c.c. was drawn with each bleeding, 15 c.c. being necessary in the analyses that were made. A control sample was always secured before an injection was made. The number of samples secured varied, but in general three or four were secured at fifteen minute intervals. In a few instances of fatal injections, only one or two samples were secured, and under these conditions blood was occasionally obtained from the heart. This is noted in the tables.

The following analyses were made as quickly as possible after the blood was drawn. The hydrogen ion concentration was estimated colorimetrically and electrometrically. In the colorimetric method, 1 c.c. of blood was dialyzed under liquid petrolatum against 0.9 per cent. sodium chlorid solution, also under liquid petrolatum. At the end of from five to ten minutes, the dialysate was treated with phenolsulphonephthalein and matched at once against standards according to the method of Levy, Rowntree and Marriott.[3] The colorimetric result was confirmed electrometrically as often as possible, and when the value appeared to be unusual or important. In the beginning of our work, we used 2.5 c.c. of blood for the electrometric measurements, but during the course of the work refinement in the apparatus and technic permitted us to use 0.5 c.c. for a large proportion of the measurements and eventually only 1 drop of blood was used. The electrometric technic has been previously described by De Eds and Hanzlik[4] and need not be repeated here.

Suffice it to say that we took special precautions to control the accuracy of the results obtained by testing the apparatus frequently with standard phthalate (p_H 3.97) and with bloods of induced asphyxia. Hemoglobin estimations were made for the purpose of observing changes in blood volume, using 0.5 c.c. of blood diluted to 1 per cent. solution according to Palmer's[5] technic. Carbon dioxid content, and capacity (alkaline reserve) were estimated in 5 c.c. of plasma (centrifugalized and kept under liquid petrolatum) according to Van Slyke and Cullen's method.[6] Urea-nitrogen was estimated by the Van Slyke-Cullen modification[7] of the urease-aeration method of Marshall, using 1 c.c. of blood. Ammonia was estimated according to Folin's[8] aeration method in 1.5 c.c. of blood, many of the estimations having been made on samples that had been used previously for the electrometric estimations of p_H. For the ammonia, the sacrifice of blood was as small as possible, owing to the uncertain significance of small changes in its content. Lactic acid was estimated in 5 c.c. of blood colorimetrically with the aid of a Benedict colorimeter according to Harrop's[9] method, using zinc lactate as the standard. Precipitation with 5 per cent. copper sulphate solution was found to be most suitable for dog blood. The addition of 0.05 mg.

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3. Levy, R. L.; Rowntree, L. G., and Marriott, W. M.: A Simple Method for Determining Variations in the Hydrogen Ion Concentration of the Blood, Arch. Int. Med. **16**:389 (Sept.) 1915.

4. De Eds, F., and Hanzlik, P. J.: J. Biol. Chem. **60**:355 (June) 1924.

5. Palmer, W. W.: J. Biol. Chem. **33**:119 (Jan.) 1918.

6. Van Slyke, D. D., and Cullen, G. E.: J. Biol. Chem. **30**:289 (June) 1917.

7. Van Slyke, D. D., and Cullen, G. E.: A Permanent Preparation of Urease, and Its Use for Rapid and Accurate Determination of Urea, J. A. M. A. **62**:1558 (May 16) 1914.

8. Folin, O.: A Laboratory Manual of Biologic Chemistry, D. Appleton & Co., 1916.

9. Harrop: Proc. Soc. Exper. Biol. & Med. **17**:162, 1920.

of lactic acid to old serum was found to be the limit of sensitivity; 0.1 gm. was readily detected and 0.5 mg. in 5 c.c., equivalent to 0.01 per cent. concentration, gave a deep rose color. The normal lactic acid content of blood ranges from about 0.029 to 0.1 per cent., i. e., from 1 to 5 mg. in 5 c.c. of blood.

The method, therefore, was sufficiently accurate for our purpose and decidedly advantageous over the time consuming extraction methods. Dextrose, phenol, acetic acid, alcohol and agar in blood gave negative tests with the reagents used in the method. Acacia gave a very slight pinkish yellow or brown and therefore not the characteristic color change. Hence, it appeared that these agents as constituents of the solutions that were injected did not contribute to the increases in lactic acid content when such were found. Total phosphate was estimated in 2.5 c.c. of blood according to the method of Bell and Doisy.[10] Sugar was determined in 2 c.c. of blood by the picramic method of Benedict according to the technic of Myers and Bailey.[11]

The sedimentation rate of corpuscles was obtained by comparing the column of corpuscles that separated from the plasma in a given time with the control samples of blood. This was done in both large and small test tubes. Frequently this phenomenon was very striking in comparison with the control tubes and merely required note of the fact. There were variations, of course, and under our conditions time was not available to evaluate these properly; the results are, therefore, recorded only with respect to an increase in rate of sedimentation or its absence. Almost invariably an increase in sedimentation rate was accompanied by agglutination, which could be recognized frequently in the supernatant plasma layer or in the suspension of a thin column of plasma in a small bore pipet. In doubtful cases microscopic examinations were made. Hemolysis was recognized from direct inspection of the plasma, and darkening of the blood by comparison with the control.

The important results and effects that were observed are recorded in the tables. A few tracing records of blood pressure and respiration are introduced to illustrate the changes in these functions. Physiologic analyses of these changes were not made as we were interested, for the time, in chemical changes. The effects of several of the agents in dogs are recorded for the first time. The extraordinary and unsuspected effects of some of the relatively inert agents on blood pressure and respiration, notably agar in low concentration, deserve further study. In several instances, the agents or solutions were used in high enough dosage or concentration to produce definite effects; so any effects short of definite ones produced by lower dosage merely emphasize the desirability of these chemical studies, which were made in order to reveal subtler changes that might be fundamental and produce moderate symptomatic changes, both subjective and objective and of importance clinically. The details of all results are discussed according to groups of agents. However, before proceeding to this, the changes from bleeding and asphyxia will be considered, since bleeding was a part of the routine in securing blood samples and is known to produce changes in blood composition. Asphyxia was used as a method of controlling changes in p_H, carbon dioxid, etc.

10. Bell, R. D., and Doisy, E. A.: J. Biol. Chem. **44**:55 (Oct.) 1920.
11. Myers, V. C., and Bailey, C. B.: J. Biol. Chem. **24**:147 (Feb.) 1916.

TABLE 1.—*Effects of Repeated Bleeding on Blood Composition and Symptoms* *

Volume of Blood Drawn	Time; End of Min.	Hemoglobin, per Cent.	p_H Colorimetric	CO_2 Content CO_2 Capacity Per Cent. by Volume	Urea Nitrogen, Mg. in 100 C.c.	Lactic Acid, per Cent.	Sugar, per Cent.	Total Phosphate, Mg. in 100 C.c.	Blood Pressure, Mg. Mercury	Pulse Rate	Respiratory Rate	Rectal Temperature, C.	General Symptoms	Sedimentation Rate	Agglutination and Other Changes
Experiment 31, Dog Weighing 6.5 Kg., One-Half Dose															
20	...	100	7.25	46.5/51.5	10.2	0.21	0.15	6.93	110	96	48	37			
15	30	98	7.2	45.3/49.4	10.0	0.22	0.14	5.04	106	58	30	....			
15	60	97	7.25	46.0/49.2	9.1	0.23	0.14	5.12	106	100	36	36			
15	90	102.6	7.3	45.1/48.2	10.0	0.23	0.13	4.82	108	100	36	35.5	None	Unchanged	None
15	120	99.0	7.3	44.2/46.2	10.5	0.21	0.15	5.25	104	...	..	....			
15	150	98.5	7.3	42.8/44.3	9.0	0.19	0.17	5.47	90	132	48	34.6			
Experiment 33, Dog Weighing 24.3 Kg., One-Half Dose															
15	...	100	7.1	50.0/51.2	11.2	0.14	0.07	3.5	90	96	60	....			
15	30	106.4	7.1	51.2/52.9	10.3	0.15	0.071	3.3	100	...	..	....			
15	60	111.1	7.15	51.2/52.9	10.8	0.17	0.081	3.4	100	100	64	....			
15	90	109.3	7.1	51.2/52.9	10.6	0.20	0.078	3.5	100	110	64	....	None	Unchanged	None
15	120	114.9	7.15	51.2/52.9	11.6	0.22	0.075	2.96	100	...	..	....			

* In the tables one-half dose equals one-half the full dose of morphin, the full dose being 0.5 c.c. of 4 per cent. solution per kilogram subcutaneously. In all cases in which no record is made it is understood that the dog received a full dose of morphin. The sign + means present or increase; —, absence or decrease; H, hemolysis; D, darkening; art. resp., artificial respiration; oc, occasional; Ag, agglutination; A, after; B, before; ?, doubtful or questionable, and tr, trace.

BLEEDING AND ASPHYXIA

It can be stated at once that repeated bleeding as practiced in the experiments and to the end of two hours was not responsible for the results with the different agents that were injected. This is adequately illustrated by the results of Experiments 31 and 32 on two morphinized dogs presented in Table 1. The small variations in the changes pertaining to the blood constituents, including those for hemoglobin and carbon dioxid in Experiment 31, are considered to be within the range of experimental error. Although there was considerable accuracy in the analytic methods employed, under the conditions, the changes of 5 per cent., plus or minus, were generally disregarded, though they might be of significance in a particular case. Our conclusions are based on more pronounced changes. The changes and irregularities in blood pressure, pulse and respiratory rates during bleeding alone were too small to be of any significance. The rectal temperature of the one dog (Experiment 31) that was observed fell 2.4 degrees C. This is the tendency, however, in morphinized dogs.

The effects of asphyxia were observed in ten dogs, usually at the end of an experiment. Reproduction of the tabulation would serve no useful purpose because the changes observed were those that are well known; namely, increase in carbon dioxid and change of p_H toward acidity, these being the most important for our purpose. Illustrations of asphyxial changes are given in Table 2 under hexamethylenamin 4.7 per cent., and in Table 3 under 10 per cent. sodium chlorid (Dog 11), under 40 per cent. hexamethylenamin (Dog 5), and under 85 per cent. sucrose (Dog 10). In addition to these changes there were darkening of the blood, increase in lactic acid as a rule, and the usual changes in blood pressure, respiration and pulse rate. The asphyxia was induced by attaching a long rubber tube to the tracheal cannula. As far as lactic acid is concerned, Macleod [12] reported this not to be increased in blood when venous at the end of asphyxia, and that only about one-half the amount was usually present (0.019 per cent.). On the other hand, he demonstrated an excess of blood lactic acid in anoxemia but not in surgical shock.

It is concluded that the repeated bleeding of small quantities of blood as practiced in our experiments produced no important changes in blood composition; therefore, this routine procedure was a negligible factor in the changes produced by the intravenous injection of the various agents that were studied. Asphyxia always produced definite changes in the appearance and reaction (p_H) and carbon dioxid content of the blood, and also some other changes. It served the purpose of a useful control of changes produced in the same direction by various agents in the absence of induced asphyxia.

12. Macleod, J. J. R.: Am. J. Physiol. **55**:184 (March) 1921.

BALANCED AND ISOTONIC SOLUTIONS

The solutions used were 0.9 and 1 per cent. sodium chlorid solution (five dogs), Tyrode's solution (one dog), and 4.7 per cent. solution of hexamethylenamin (two dogs). The results are presented in Table 2.

Isotonic Sodium Chlorid and Tyrode's Solutions.—With the sodium chlorid solutions, the principal effects consisted of variable blood dilution, roughly proportional to the volume injected; diminution in carbon dioxid; a slight and variable tendency toward acidity (lowering of p_H values), and very small increases in blood pressure and pulse rate in the majority of dogs. The fall in temperature was also slight, ranging from no change to 1.4 degrees C. When diminution in carbon dioxid occurred, this change could be accounted for by dilution of the blood. Tyrode's solution produced effects similar to those of sodium chlorid, except for the marked change in p_H at the end of thirty minutes. This low value of 7.1, however, was not confirmed electrometrically. A similar value was found in one out of five dogs receiving sodium chlorid solution.

Hexamethylenamin, 4.7 Per Cent. Solution.—This approximately isotonic solution of hexamethylenamin produced more marked changes in p_H toward acidity than the sodium chlorid solutions, the changes in blood dilution and blood pressure being similar to those after sodium chlorid. Carbon dioxid estimations were not made after hexamethylenamin. From this it would appear that hexamethylenamin, as a drug, had a more pronounced effect than sodium chlorid. Further on it will be seen that higher concentrations of hexamethylenamin produced even more marked effects.

The results with 0.9 and 1 per cent. sodium chlorid and Tyrode's solutions indicate that these solutions, though isotonic and balanced, nevertheless may alter the blood and higher functions when they are injected intravenously. Therefore, they are not strictly physiologic, as would be expected from the considerable differences in their composition from that of blood, and as has been previously pointed out by physiologists. According to the recent report of Blumenstock and Luckhardt,[13] 0.9 per cent. sodium chlorid solution was the least physiologic solution of the three solutions used intravenously, namely, Ringer's, calcium-free Ringer's and 0.9 per cent. sodium chlorid. Nine-tenths per cent. sodium chlorid solution caused the most vigorous diuresis and the highest elimination of nitrogen. It often caused shivering. Fujimaki [14] reports that the intravenous injection of from 20 to 25 c.c. of 0.9 per cent. sodium chlorid solution daily into rabbits causes an increase in rectal temperature and of the carbon dioxid of the blood.

13. Blumenstock, J., and Luckhardt, A. B.: Am. J. Physiol. **67**:514 (Feb.) 1924.

14. Fujimaki: Arch. f. exper. Path. u. Pharmakol. **103**:178, 1924.

TABLE 2.—*Effects of Sodium Chlorid (0.9 and 1 Per Cent.), Tyrode's Solution and Hexamethylenamin (4.7 Per Cent.) on Blood* *

Dog No. and Weight (Kg.)	Dose per Kilogram	Time of Blood Specimens in Minutes	Hemoglobin, per Cent.	p_H Colorimetric	p_H Electrometric	CO_2 Content / CO_2 Capacity Per Cent. by Volume	Urea Nitrogen, Mg. in 100 C.c.	Lactic Acid, per Cent.	Sugar, per Cent.†	Blood Pressure, Mm. Mercury	Pulse Rate	Respiratory Rate	Rectal Temperature C.	General Symptoms	Sedimentation Rate	Agglutination and Other Changes
Sodium Chlorid, 0.9 and 1 per Cent. Solution																
12 (10.2)	10 c.c.	B	100	7.2+	7.33	..	12.9	..	....	116	72	21	36	.....	.....	.....
		22 A	91.3	7.3	7.33	..	15.4	..	....	124	78	20	35.6	None	.....	.....
		44 A	90.9	7.25	7.23	..	12.9	..	....	128	66	20	35.6			
13 (9.4)	3.9 c.c.	B	100	7.3	7.20	58‡	....	None	0.12	106	66	18	36.3	.....	.....	.....
		7 A	75.7	7.0	7.10	63	....	..	....	110	64	18	36.0	.....	.....	.....
		20 A	74.9	7.0	7.12	..	....	..	....	110	64	20	35.9	.....	.....	.....
		35 A	79.7	7.15	7.15	..	....	+	0.11	116	64	18	35.7	.....	.....	.....
		75 A	80.3	7.1	....	68	....	..	....	124	90	18	35.4	.....	.....	.....
		145 A	93.0	7.1	7.1	59	....	+ (tr.)	0.12	116	120	18	35.6	.....	.....	.....
14 (6.6)	7.6 c.c.	B	100	7.3	7.3	63/65	16.1	..	....	120	96	30	37.6	.....	.....	.....
		5 A	90.9	7.25	7.21	63/68	16.2	..	....	86	96	27	37.0	.....	.....	.....
		34 A	97.5	7.3	....	59/59	....	..	....	60	132	30	36.6	.....	.....	.....
		72 A	89.7	7.35	7.36	59/54	15.4	..	....	50	144	...	36.4	.....	.....	.....
15 (13.1)	7.6 c.c.	B	100	7.25	7.31	64/65	10.9	+	0.1	110	54	20	37.0	.....	.....	.....
		11 A	102	7.2	....	60/65	12.4	..	....	110	60	14	36.0	.....	.....	.....
		28 A	91.3	7.2	7.21	62/64	10.8	+	....	110	60	...	35.6	.....	.....	.....

									Ammonia								
17 (6.1)	32.8 c.c.	B	100	6.9	7.18	36/46	13.6	+	+	100 140 6 min. later	104	150	37.6	Asphyxial struggling	None	None	
No morphin (1% sodium chlorid solution)		10 A	81.8	7.3	7.16	37/42	11.8	..	....	120	168	...	37.0	None			
		34 A	88.9	7.0	7.26	34/46	12.2	++	None	70	110	120	37.0				
Tyrode's Solution																	
									Ammonia								
21 (6.1) ½ dose	16.4 c.c.	B	100	7.35	....	59/62	11.3	+++	+++	40	72	24	33.6	None	None	None	
		15 A	88.5	7.4	....	55/59	11.9	..	....	60	96	...	....				
		15 A	85.1	7.1	....	52/58	11.5	..	+++	56	94	...	....				
	16.4 c.c.	55 A	....	7.2	....	52/58	12.5	+	....	60	94	...	31.8				
Hexamethylenamin, 4.7 per Cent. Solution																	
4 (9.9)	10.1 c.c.	B	100	7.3	....	..	11.8	..	....	90	...	...	....	.....	.....	.....	
		18 A	96.5	7.3	....	..	13.7	..	....	104	...	...	....	.....	.....	.....	
		37 A	96.5	7.2	....	..	8.1	..	....	110	...	..	....	.....	.....	.....	
		60 A	94.0	7.1	....	..	10.9	..	....	110	...	...	....	Struggling	.....	D	
		75 A	90.0	7.1	....	..	11.8	..	....	120	...	Deep and irregular	....	Struggling	.....	D	
9 (10.4)	9.6 c.c.	B	100	7.25	7.23	..	16.5	..	....	130	...	...	36.8	.....	.....	.....	
		35 A	83.7	7.2	7.17	..	17.5	..	....	126	...	...	37.8	.....	.....	.....	
No morphin	Asphyxia	10 A	84.3	6.9	6.94	..	15.0	..	....	No record	...	...	37.2	.....	.....	.....	
	Art. resp.	15 A	91.7	7.1	7.23	..	17.4	..	....	No record	...	Art. resp.	....	.....	.....	.....	

* Phosphates were not estimated.

† Data headed ammonia in this column in all tables means changes in ammonia in milligrams per hundred cubic centimeters of the blood, when the quantity is stated; all other data pertain to sugar.

‡ The carbon dioxid data in this experiment mean total carbon dioxid content only.

The increase in carbon dioxid is interpreted as alkali reserve, the alkali being called out in response to an increase in acidity. No observations of p_H were made, however. Such moderate disturbances in ionic equilibrium and state of the blood colloids as may be brought about by these solutions can conceivably account for alterations in functions. On the whole, therefore, when used in high dosage these solutions cannot be regarded as altogether harmless. However, the very small quantities used by us as solvents or vehicles for the various agents that were injected could not be responsible for the marked effects that were observed. They were the most practical vehicles for our use, whole blood, no doubt, being superior.

It is concluded that 0.9 and 1 per cent. sodium chlorid solution and Tyrode's solutions when injected intravenously in doses of from 3.9 to 32.8 c.c. per kilogram cause dilution of the blood with a tendency to diminution of its carbon dioxid content, sometimes considerable change toward acidity (lowering of p_H), and the usual moderate increases in blood pressure and pulse rate. In small dosage, the effects are practically negligible. A solution of 4.7 per cent. (isotonic) hexamethylenamin also produced dilution and a greater tendency to acidity of the blood.

HYPERTONIC SOLUTIONS

The solutions studied were sodium chlorid, 10 per cent., hexathylenamin, 40 per cent., sucrose, 85 per cent., dextrose, 50 per cent., and urea, 18 per cent. These solutions have all been injected intravenously in human subjects for various purposes. Certain ones have been exploited by Stejskal [15] under the name of "osmotherapy." It is stated by Stejskal that the noninjuriousness of the following solutions and doses has been established for human subjects: from 10 to 20 c.c. of 10 per cent. sodium chlorid solution; from 20 to 100 c.c. of 50 per cent. dextrose solution; 20 c.c. of 50 per cent. sucrose solution; 20 c.c. of from 10 to 20 per cent. sodium sulphate solution; 20 c.c. of 20 per cent. urea solution and from 10 to 20 c.c. of 10 per cent. calcium chlorid solution. However, the enthusiastic claims of this author are not convincing. One hundred cubic centimeters of 10 per cent. sodium chlorid solution has been injected in patients by Velden [16] who states that it produces shortening of the coagulation time of blood with beneficial results in hemorrhage. Schenk [17] states that he has confirmed this phenomenon with hypertonic salt solution and observed it with calcium chlorid. Blümel [18] makes the same claim for oral administration of salt,

15. Stejskal: Grundlagen der Osmotherapie, Vienna, 1922, p. 215.
16. Velden: Deutsche med. Wchnschr. **35**:197, 1909; Zentralbl. f Herzkrankh. u. Gefässkrankh. **11**:61, 1919.
17. Schenk: Ztschr. f. d. ges. exper. Med. **11**:166, 1920.
18. Blümel: Med. Klin. **2**:1175, 1910.

and Neuhof and Hirshfeld [19] assert that intravenous administration of sodium citrate produces the same effect. The basis for the intravenous use of hypertonic sodium chlorid solution appears to be that tissue juice is drawn into blood along with tissue fluid in general. Intravenous injections of 200 c.c. of 15 per cent. sodium chlorid solution in man have been practiced by Wynn; [20] of 15 and 30 per cent. sodium chlorid solution by Cushing and Foley [21] and by Sachs and Belcher.[22] Barach, Mason and Jones [23] studied certain of blood changes caused by 15 per cent. sodium chlorid solution in patients and rabbits. The venous oxygen in the three patients studied was markedly lowered, and the carbon dioxid content showed no constant or decided changes. Other changes were increase in blood or plasma volume. Chlorid content was constant in patients, but persistently increased in rabbits; fragility of corpuscles was not impaired, and one patient showed hemolysis. Various claims for and uses of 50 per cent. dextrose solution (up to 1 gm. per kilogram) will be found in papers by Bürger and Hagemann,[24] Wells and Blankinship, [25] Litchfield,[26] Sansum [27] and Lo Monaco.[28] The great variety of effects, including blood changes, in animals that have been observed by many investigators will be found in the textbooks of pharmacology by Sollmann,[29] and by Meyer and Gottlieb,[30] who think that the nature of cell injury after hypertonic solutions is not understood and that chemical decompositions and cleavages occur. The results obtained by us on dogs are presented in Table 3.

Sodium Chlorid, 10 Per Cent. Solution.—The changes in blood produced by 10 per cent. sodium chlorid solution were characterized by

19. Neuhof, H., and Hirshfeld, S.: New York M. J. **133**:95 (Jan. 15) 1921.
20. Wynn, J.: Intravenous Injections of Hypertonic Salt Solutions in Neurosyphilis, Arch. Int. Med. **29**:72 (Jan.) 1922.
21. Cushing and Foley: Proc. Soc. Exper. Biol. & Med. **17**:217, 1920.
22. Sachs, E., and Belcher, G. W.: Use of Saturated Salt Solution Intravenously During Intracranial Operations, Preliminary Report, J. A. M. A. **75**:667 (Sept. 4) 1920.
23. Barach, A. L.; Mason, W., and Jones, B. P.: Effect of Administration of Hypertonic Salt Solution on Blood Volume and Certain Related Blood Constituents, Arch. Int. Med. **30**:668 (Nov.) 1922.
24. Bürger, M., and Hagemann, E.: Deutsch. med. Wchnschr. **47**:207 (Feb. 24) 1921.
25. Wells, C. W., and Blankinship, R. C.: Intravenous Injections of Hypertonic Glucose Solution in Influenzal Pneumonia, J. A. M. A. **74**:75 (Jan. 10) 1920.
26. Litchfield, W. F.: Intravenous Glucose Injections in Shock, J. A. M. A. **69**:1410 (Oct. 27) 1917.
27. Sansum, W. D.: Glucose Injections, J. A. M. A. **68**:1885 (June 23) 1917.
28. Lo Monaco: Gior. di med. mil. **66**:176, 1918; Gas. med. lomb., Milan, **76**:97, 1918; Rassegna di clin. terap. **17**:73, 1918; Presse méd. **26**:617, 1918.
29. Sollmann: Manual of Pharmacology, Ed. 2, W. B. Saunders Company, 1923.
30. Meyer and Gottlieb: Experimentelle Pharmakologie, Ed. 4, 1920, p. 441.

TABLE 3.—*Effects of Sodium Chlorid (10 Per Cent.), Hexamethylenamin (40 Per Cent.), Sucrose (85 Per Cent.), Dextrose (50 Per Cent.), Urea (18 Per Cent.), and Sodium Chlorid (0.1 Per Cent.)*

Dog No. and Weight (Kg.)	Dose per Kilogram	Time of Blood Specimens in Minutes	Hemoglobin, per Cent.	p_H Colorimetric	p_H Electrometric	CO_2 Content CO_2 Capacity Per Cent. by Volume	Urea Nitrogen, Mg. in 100 C.c.	Lactic Acid, per Cent.	Sugar, per Cent.†	Blood Pressure, Mm. Mercury	Pulse Rate	Respiratory Rate	Rectal Temperature C.	General Symptoms	Sedimentation Rate	Agglutination and Other Changes
Sodium Chlorid, 10 per Cent. Solution																
3 (7.4)	.....	B	100	7.4	....	..	9.0	..	....	120	...	...	....	.....	.....	.....
	3.4 c.c.															
No morphin		A	87.3	7.1	....	..	10.6	..	....	116	...	...	....	.....	.....	.....
			85.1	7.3	....	..	10.9	..	....	104	...	..	....	.....	.....	.....
11 (19)	.....	B	100	7.2	7.2	..	11.5	..	....	136	78	240	36.8	.....	.....	.....
	9.1 c.c. preceded by dextrose, 100 c.c.															
		9 A	82.3	7.2	....	..	13 3	..	....	154	180	240	....	.....	.....	.....
		17 A	85.1	7.2	6.79	..	11.7	..	.. .	150	120	228	36.3	.....	.....	.....
		36 A	85.1	7.2	....	..	12.7	..	....	140	84	...	36.6	.....	.....	.....
		64 A	104.2	7.2	....	..	11.3	..	....	134	78	120	36.6	.....	.....	.....
	9.1 c.c.															
		13 A	76.6	7.3	....	..	9.5	..	....	146	85	...	....	.....	.....	.....
		23 A	75.2	7.1	6.77	..	12.7	..	....	140	130	40	....	Tremors, struggling, twitching	+	H
		49 A	....	....	7.2+	..	11.6	..	....	130	108	...	....	Increased reflex excitability		
		65 A	82.7	7.3	....	..	13.8	..	....	130	..	...	37.5	Tremors marked	+	H
	Asphyxia	6 A	83.7	7.1	6.75	..	10.3	..	....	134	...	...	....	Convulsions	..	..
15 (13.1)	.....	B	91.3	7.20	7.21	62/64	10.8	+	0.1	110	60	14	35.6	.....	.....	.....
	7.6 c.c. preceded by 0.9 per cent. sodium chlorid															
		10 A	86.6	7.15	7.11	52/56	11.4	+	....	140	112	...	34.2	.....	.....	.....
		30 A	85.8	7.0	7.03	51/54	11.4	++	0.1	140	...	..	....	Twitching, tremors	.....	.....
		63 A	84.7	7.1	7.09	52/65	12.5	..	....	130	92	18	34.2	Twitching	.....	.....
		98 A	89.7	7.1	7.17	50/56	11.2	++	0.09	120	60	18	34.0	.....	.....	.....

									Ammonia							
18	.....	B	....	6.8	7.04	54.9	12.5	+	+	60	175	36	36.2	.....	.....	.....
(8.2)	6.1 c.c. preceded by 6 per cent. acacia, 16 c.c					62.3										
		30 A	98.5	6.9	7.04	53.1	12.1	++	....	84	144	28	35.6	Convulsions, tremors	+	H
						56.8										
		68 A	....	6.9	7.0	51.2	13.3	++	++	80	158	30	35.2	.....	+	H
						54.9										
									Hexamethylenamin, 40 per Cent. Solution							
5	....	B	100	7.4	....	..	8.5	..	....	120	78	18	36.4	.....	.....	.....
(7.2)	3.5 c.c.															
	1.39 gm.	15 A	92.6	7.1	....	..	9.8	..	....	140	90	16	35.6	Struggling	.....	.....
		30 A	100	7.2	....	..	10.9	..	....	140	78	...	35.5	.....	.....	.....
	3.5 c.c.															
	1.39 gm.	18 A	93.6	6.7	....	..	11.5	..	....	120	90	16+ deep	....	Twitching in legs	.....	.....
		22 A	88.9	7.3	....	..	10.6	..	....	120	...	...	....	.....	.....	.....
	Asphyxia	15 A	80.4	6.8	....	..	11.1	..	....	60	...	...	35.2	Tremors; struggling	.....	.....
14	.....	B	89.7	7.35	7.36	54	15.4	..	....	40	138	...	36.0	.....	.....	.....
(6.6)	7.6 preceded by 50 c.c. sodium chlorid 10 per cent.															
	3.03 gm.	12 A	76.0	7.1	7.02	44	13.8	..	....	66	132	...	35.6	.....	.....	.....
						50										
		30 A	73.2	7.0	7.0	44	15.8	..	....	80	120	...	35.4	Tremors	.....	.....
						50										
		60 A	70.7	6.9	7.0	45	14.9	..	....	0	...	...	....	.....	.....	.....
						50										
	Art. resp.	69 A	63.7	6.9+	6.97	32	14.6	..	....	26	72	Art. resp.	34.9	Resuscitation with epinephrin	.....	.....
						37										
		89 A	56.0	6.9	6.88	29	16.6	..	....	0	42	...	34.6	Collapse; died	.....	.....
						31										

TABLE 3.—*Effects of Sodium Chlorid (10 Per Cent.), Hexamethylenamin (40 Per Cent.), Sucrose (85 Per Cent.), Dextrose (50 Per Cent.), Urea (18 Per Cent.), and Sodium Chlorid (0.1 Per Cent.).—(Continued)*

Dog No. and Weight (Kg.)	Dose per Kilogram	Time of Blood Specimens in Minutes	Hemoglobin, per Cent.	p_H Colorimetric	p_H Electrometric	CO_2 Content / CO_2 Capacity Per Cent. by Volume	Urea Nitrogen, Mg. in 100 C.c.	Lactic Acid, per Cent.	Sugar, per Cent.†	Blood Pressure, Mm. Mercury	Pulse Rate	Respiratory Rate	Rectal Temperature C.	General Symptoms	Sedimentation Rate	Agglutination and Other Changes
15 (13.1)	.....	B	.....	7.2	7.28	43/47	10.3	..	....	120	60	16	33.8	.....	.....	.....
	6.6 c.c. preceded by 200 c.c. 0.9 per cent. sodium chlorid and 100 c.c. 50 per cent. dextrose									70 (6 minutes after injection)						
	2.66 gm.	16 A	.....	7.0	6.86	40/48	11.5	..	....	110	120	36 deep, irregular	33.4	Tremors, shivering	.....	.....
		30 A	.....	7.2	6.75	36/43	11.5	..	....	110	...	...	....	Continuous tremors	.....	.....
		60 A	.....	7.0	6.88	35/45	12.0	..	....	110	120	18 very deep, irregular	33.6	.....	.....	.....
16 (5.1)	..... 9.8 c.c.	B	100	7.0	6.98	32/41	12.5	..	....	100	228	...	37.4	.....	.....	.....
No morphin	3.9 gm.	10 A	77.5	6.9	6.89	37/40	14.6	..	....	40	156	21	36.4	.....	.....	.....
		24 A	77.8	6.8	6.8	30/33	14.6	..	....	70	144	21	36.4	Collapse	.....	.....
	Art. resp.	89 A	81.5	6.8	6.65	24/33	13.8	..	....	40	72	Art. resp.	34.1	Struggling; twitching	.....	H
						Sucrose, 85 per Cent. Solution										
8 (8.6)	..... 5.8 c.c.;	B	100	7.3	....	..	11.1	..	....	94	60	20	37.0	.....	.....	.....
No morphin	5.26 gm.	10 A	78.4	7.3	....	..	11.4	..	....	120	78	Increased; deepened	36.5	Urination	.....	.....
		29 A	102.6	7.3	....	..	....	..	....	114	70	...	36.6	.....	.....	.....
		168 A	112.4	7.1	....	..	8.4	..	....	110	120	24	37.2	.....	.....	.....
	5.8 c.c.; 5.26 gm.	10 A	88.5	7.3	....	..	9.7	..	....	120 124	156	...	37.5	.....	.....	.....
		17 A	93.0	7.3	....	..	10.5	..	....	124	144	...	37.0	Twitching	.....	.....
		30 A	....	7.3	....	..	9.7	..	....	110	...	...	....	.....	.....	.....

10 (12.8)	.....	B	99.6	7.3	7.32	..	9.8	..	....	120	132	12	36.0	.....	.....	.....
	3.9 c.c. preceded by 50 c.c. hexamethylenamin, 40 per cent.															
	3.32 gm.	5 A	67.6	7.3	....	..	8.3	..	....	110	192	6	35.6	Urination	.....	.....
		17 A	83.3	7.1	....	..	10.0	..	....	120	160	Deep Deep	35.6	Twitching, struggling	.....	D
		29 A	95.2	7.3	7.31	..	9.5	..	....	130	...	10	....	.....	.....	.....
		47 A	94.8	7.2	7.22	..	9.5	..	....	130	...	...	35.6	.....	.....	.....
	Asphyxia induced	11 A	97.6	7.0	6.90	..	8.6	..	....	160	...	Tube to trachea	....	.....	.....	.....
		18 A	96.2	6.9	6.86	..	9.5	..	....	80	...	...	....	Died	.....	.....
							Dextrose, 50 per Cent. Solution									
11 (19.0)	.....	B	100	7.0	7.0	..	14.7	..	....	134	116	...	37.8	.....	.....	.....
	5.26 c.c.; 2.63 gm.	8 A	97.1	7.2	....	..	13.3	..	....	128	186	...	....	.....	.....	.....
		16 A	99.5	7.2+	6.93	..	15.0	..	....	134	168	...	37.4	.....	.....	.....
		32 A	102.5	7.0	7.02	..	12.5	..	....	140	136	...	37.4	.....	.....	.....
		60 A	100.5	7.0	6.89	..	14.5	..	....	134	96	...	37.4	.....	.....	D
		115 A	71.7?	7.2+	....	..	13.9	..	....	130	84	...	37.2	.....	.....	.....
		171 A	70.9?	7.2	....	..	11.5	..	....	130	78	...	36.8	.....	.....	.....
13 (9.4)	.....	B	93.0	7.1	7.1	59*	....	..	....	110	120	18	35.6	.....	.....	.....
	5.3 c.c.; 2.66 gm. preceded by 50 c.c. sodium chlorid, 0.9 per cent.															
		9 A	61.5	7.3	....	56	....	..	....	126	120	16	35.0	.....	.....	.....
		24 A	76.6	7.2	....	56	....	..	....	116	80	16	35.0	.....	.....	.....
		64 A	84.0	7.1	....	..	....	..	....	124	100	20	35.4	.....	.....	.....
15 (13.1)	.....	B	89.7	7.1	7.16	50/56	11.2	..	....	120	60	18	34.0	.....	.....	.....
	7.63 c.c. preceded by 98.8 c.c. sodium chlorid, 10 per cent.															
	3.82 gm.	16 A	75.7	7.1	7.11	52/56	11.5	..	....	130	78	...	33.2	Twitching	.....	.....
		29 A	86.6	7.1	7.08	48/54	9.3	..	....	114	57	...	33.4	Tremors	.....	.....
		60 A	....	7.0	6.96	42/49	10.9	..	....	114	58	16	33.8	.....	+	.....
		120 A	....	7.2	7.23	43/47	10.3	..	....	114	60	...	33.8	.....	+	.....

* Figures represent carbon dioxid capacity of plasma.

TABLE 3.—*Effects of Sodium Chlorid (10 Per Cent.), Hexamethylenamin (40 Per Cent.), Sucrose (85 Per Cent.), Dextrose (50 Per Cent.), Urea (18 Per Cent.), and Sodium Chlorid (0.1 Per Cent.).—(Continued)*

Dog No. and Weight (Kg.)	Dose per Kilogram	Time of Blood Specimens in Minutes	Hemoglobin, per Cent.	p_H Colorimetric	p_H Electrometric	CO_2 Content / CO_2 Capacity Per Cent. by Volume	Urea Nitrogen, Mg. in 100 C.c.	Lactic Acid, per Cent.	Sugar, per Cent.†	Blood Pressure, Mm. Mercury	Pulse Rate	Respiratory Rate	Rectal Temperature C.	General Symptoms	Sedimentation Rate	Agglutination and Other Changes
Urea, 18 per Cent. Solution																
16 (5.1)	.....	B	81.5	6.8	6.66	24/33	13.8	..	....	40	72	Art. resp.	36.4	.....	.....	.....
	9.8 c.c. preceded by 50 c.c. hexamethylenamin, 40 per cent. 1.76 gm.	17 A	59.3	6.7	6.61	24/44	13.7	..	...	40	108	Spontaneous respiration	34.1	Twitching	.....	H
											60	Art. resp.		Struggling		
		30 A	60.1	6.9	6.56	27/32	12.9	..	....	44	60	...	32.8	Quiet	.....	H
		65 A	51.1	6.6	6.59	27/30	....	..	....	20	48	...	....	.....	+ marked	H
19 (13.2)	....	B	96	7.0	7.09	72/79	11.1	++	++	120	70	20	36.2	.....	+	H; D
										170 (20 minutes after injection)						
	7:58 c.c.; 1.36 gm. preceded by 16 c.c. per kilogram of 6 per cent. acacia in 0.9 per cent. sodium chlorid solution										180	36 Deepened; irregular	35.6			
		33 A	90	....	6.62	72/74	10.3	..	+	150	114	18 Deep Irregular	35.4	Oc. twitching	+ marked	More H
		60 A	93	6.9	7.05	70/74	10.9	+	....	140	60	...	35.2	.....	+ marked	Still more H
Sodium Chlorid, 0.1 per Cent. Solution																
21 (6.1)	.....	B	77.8	7.4	....	52/57	11.1	+	+	50	60 52	...	29.8	.....	.....	H
½ dose	16 c.c. preceded by 100 c.c. horse serum	31 A	71.7	7.2	....	56/51	....	..	+	60	47	...	29.2	Fascicular twitching of leg muscles	.....	H marked
		60 A	67.5	7.2	....	53/60	12.2	++	++	50	48	...	....	Struggling	No sedimentation, but marked foaming	H marked
		74 A	62.6	7.3	....	54/60	11.5	++	...	40	40	...	28.3	.....	.....	H marked

considerable blood dilution, a tendency toward acidity (lowered p_H), and a reduction of carbon dioxid in the majority of four, and by hemolysis and an increased sedimentation rate in two dogs. There were demonstrable increases in lactic acid in the two dogs that were observed for this constituent, an increase in ammonia in one dog and practically no change in sugar in one dog. Urea was variable. Phosphate was not estimated. Acidosis in some dogs, also leukopenia, then marked lymphocytosis and a rise in temperature, are reported by Bacon, Novy and Eppler,[31] who made intravenous injections of a hypertonic solution containing sodium chlorid, 99 gm., calcium chlorid, 2.86 gm., and potassium chlorid, 3.3 gm., in 200 c.c. of water, injecting doses of 500 c.c. in from fifteen to twenty minutes.

Symptomatically, tremors, struggling, twitching and increased reflex excitability were observed in three of the four dogs and convulsions in one. These symptoms occurred generally when the changes in blood reaction, hemolysis and increased sedimentation rate were demonstrable. Blood pressure was elevated at once after injection in three dogs and somewhat lowered in one (Dog 3); pulse rate was variable, being accelerated in two and slowed in two dogs, while the respiratory rate tended to be slowed. The effects with 10 per cent. sodium chlorid solution were practically the same whether the solution was given alone or preceded by other solutions (as in Dog 15, by 0.9 per cent. sodium chlorid solution and in Dog 18 by 6 per cent. acacia).

The symptomatic effects observed by us agree with the well known effects of hypertonic sodium chlorid solution. They are generally ascribed to dehydration, changes in osmotic pressure and in ionic balance in the tissues. These factors undoubtedly play a part, but isotonic (0.9 per cent.) sodium chlorid solution also produces physiologic changes, as indicated in the preceding section. The osmotic factor is not so prominent in isotonic saline solution, though, of course, such a solution does not possess other ions in balance necessary for the tissues. The changes in p_H observed by us indicate a disturbed balance of carbonate and phosphate ions in the blood in part, and in part alterations in diffusion phenomena and surfaces. The increased rate of sedimentation would be expected from dilution of the blood, but when it is accompanied by hemolysis, as was the case in two dogs, this phenomenon indicates some surface change in the cells. The decrease in carbon dioxid can be accounted for by dilution of the blood, but the dilution does not adequately account for the change in p_H toward acidity. The acidity may be accounted for, in part at least, by the lactic acid, which was increased in two dogs. However, the blood of the other two dogs was not tested

31. Bacon, D. K.; Novy, F. O., and Eppler, H. H.: Factors in Leukocytosis, Arch. Int. Med. **30**:229 (Aug.) 1922.

for lactic acid. Total phosphate was not estimated, and therefore the evidence for an increase of fixed acids is incomplete. Urea remained unchanged or was variable in accordance with the well known tendency of this metabolite to maintain even distribution between the blood and tissues.

As far as the other blood constituents that were studied are concerned, there is no doubt that intravenous injection of 10 per cent. sodium chlorid solution causes considerable disturbance, and it appears that the symptomatic changes are dependent on these physical and chemical changes. As far as we know, the changes in p_H and carbon dioxid have not been previously described. It will be seen later on that they can be brought in line with similar changes produced by hypertonic solutions of other nontoxic and toxic agents and with isotonic solutions and suspensions of relatively inert agents and colloids. All this indicates that the mechanism of action of these nontoxic, inert and colloidal agents is concerned with disturbances in physical and chemical mechanisms of the blood and tissues, in the same sense as those involved in the action of hypertonic sodium chlorid solution.

Hexamethylenamin, 40 Per Cent. Solution.—Nineteen injections of 40 per cent. hexamethylenamin solution were made into twelve dogs, but in order to save space the results in only four dogs are presented in Table 3. The results of all the injections were practically the same and are well typified by those presented. Qualitatively the effects produced were essentially the same as those of 10 per cent. sodium chlorid solution and the other hypertonic solutions that were injected, but quantitatively they were more pronounced. There was a greater and more lasting dilution of the blood, a greater and perhaps more lasting tendency toward acidity, more marked decrease in carbon dioxid and alkali reserve. The urea was variable. Lactic acid, sugar and phosphate were not estimated. As for symptoms, these were more pronounced than with the other hypertonic solutions. The blood pressure and pulse rate were increased in two and decreased in two dogs. Collapse supervened in two (Dogs 14 and 16), necessitating resuscitation by artificial respiration. The respiration tended to be slowed, deepened and irregular in two (Dogs 5 and 15), and in Dogs 14 and 16 respiration stopped at the end of sixty and eighty-nine minutes, respectively, and they required artificial respiration. Struggling and tremors and twitching in the legs and other parts of the body were present in all the dogs. Death occurred at the end of eighty-nine minutes after injection of the hexamethylenamin in Dog 14, which had previously received 10 per cent. sodium chlorid solution. This dog had a shock level of blood pressure before the injection of hexamethylenamin which raised it 100 per cent. temporarily. However, Dog 16, which had received no morphin or solutions prior to hexamethylenamin and had a high level of blood pressure at the start.

responded to the hexamethylenamin with a shock level of pressure and required artificial respiration. From this it appears that the hypertonic hexamethylenamin solution was responsible for the marked collapse in these two animals. The tremors, struggling and twitching were undoubtedly more pronounced with the hexamethylenamin than with the other hypertonic solutions, these symptoms occurring usually when the blood p_H values were at their lowest. Changes in agglutination, hemolysis, etc., were not consistently observed, though hemolysis was noted once at the end of the experiment in Dog 16.

All changes occurring after intravenous injection of 40 per cent. hexamethylenamin indicate that this solution is injurious. It is this concentration of the drug that has been exploited by the enthusiasts of intravenous therapy for therapeutic purposes in human subjects with complete disregard of the fact that all its ordinary urinary antiseptic effects can be secured from oral administration, owing to its rapid absorption, and urinary excretion. The disturbances produced on the intravenous injection of the drug are not due to formaldehyd liberation, despite the occurrence of a slight acidity following its injection, for the following reasons: (1) the acidity would not be high enough to cause an adequate liberation of formaldehyd; (2) the slight and momentary liberation of free formaldehyd would be rendered inert by combination with proteins and by oxidation to formic acid, and (3) actual tests made by one of us (F. D.) under optimal conditions of blood acidity during asphyxia did not reveal even a trace of free formaldehyd in the blood.[32] The cause of these changes, therefore, is the hexamethylenamin itself, and this despite the fact that the drug is nontoxic in the ordinary sense. Fundamentally, the cause of the changes from hexamethylenamin given intravenously appears to be the same as that of intravenous injections in general, irrespective of the physical and chemical nature of the agent injected.

Sucrose, 85 Per Cent. Solution.—Simple syrup (U. S. P.) was prepared from chemically pure sucrose and injected intravenously in two dogs. The results are not as complete (Table 3) as with dextrose, and in view of the less pronounced effects the experiments were not extended. Data for carbon dioxid, sugar, lactic acid and phosphates are scattering and incomplete not only with sucrose, but also with other agents in Table 3 because these were our earliest experiments.[33] Dog 8 (unmorphinized) received two successive doses of sucrose alone. Ten minutes after each injection, there was considerable blood dilution from

32. De Eds, F.: Fate of Hexamethylenamin in Body and Its Bearing on Systemic Antisepsis, Arch. Int. Med. **34**:511 (Oct.) 1924.

33. At the time the full significance of the changes we were finding was not appreciated, nor was our application of the several methods, which had to be applied simultaneously, yet satisfactory enough to give reliable results.

which there was a prompt recovery. The p_H remained practically unchanged, except at the end of 168 minutes after the first injection when it was found to be 7.1; but this was not confirmed electrometrically and may have been accidental. The urea showed unimportant variations. On the other hand, Dog 10 (unmorphinized), which had recovered from an injection of 40 per cent. hexamethylenamin, responded more markedly to about three-fifths the dose of sucrose used in Dog 8. The blood dilution was more marked and lasting and the p_H values fell steadily, indicating a tendency toward acidity. The animal was killed by asphyxia. As for symptoms, these were about the same in both dogs. Both dogs urinated copiously and showed twitchings, struggling being present in Dog 10, which had received hexamethylenamin previously. The blood pressure and pulse rate were increased. The respiration was deepened in both dogs, slowed in one and somewhat accelerated in the other. The rectal temperature fell slightly.

Dextrose, 50 Per Cent. Solution.—Of the three dogs that were injected, Dog 11 received dextrose alone; Dog 13, following injections of 0.9 per cent. sodium chlorid solution, and Dog 15, after 10 per cent. sodium chlorid solution. On the whole, the results, though incomplete, were about the same as those after sucrose alone. Temporary dilution of the blood occurred in all the dogs, this being more marked in the dogs receiving previous injections of the sodium chlorid solutions. The p_H values tended to increase (tendency toward alkalinity) or remained practically unchanged. The dilution of the blood could explain the lowered carbon dioxid content. According to Fujimaki,[34] one-half the concentration (25 per cent.) of dextrose used by us increased the carbon dioxid of the blood in rabbits. Intravenous injection of 20 per cent. dextrose was found not to increase the reserve alkali (carbon dioxid) capacity of the blood by Tatum.[35] The urea in our dogs showed minor variations as usual. Lactic acid, sugar and phosphate were not estimated. In 1893, Harley [36] reported a marked increase of lactic acid soon after injection of 10 gm. per kilogram of dextrose (50 per cent. solution) intravenously in dogs; at the same time, the glycogen of the liver and muscle were reduced and acetone appeared in the breath. According to Harley, these changes occurred when the blood sugar reached 1 per cent. of the body weight. In a second report in 1894, Harley [37] described an increase in lactic acid of the blood during the first hour and reduced tension of oxygen during the first two hours after intravenous injection of dextrose. According to him, the lactic acid would bind the sodium of the blood and result in reduction of carbon dioxid,

34. Fujimaki: Arch. f. exper. Path. u. Pharmakol. **102**:236, 1924.
35. Tatum, A. L.: J. Biol. Chem. **41**:59 (Jan.) 1920.
36. Harley: DuBois, Arch. f. Physiol. Supplement, p. 46, 1893.
37. Harley: DuBois, Arch. f. Physiol., p. 451, 1894.

which would be eliminated by the increased respiration. Besides an increase in respiration, Harley observed fibrillary twitching, vomiting, motor depression and contracted pupils. On the following day the behavior was normal. In 1917 Macleod and Fulk [38] reported an increase in hydrogen ion concentration of blood after injections of dextrose. Hyperglycemia and acidosis frequently go together. The symptoms observed by us were mild, showing only small and temporary increases in blood pressure and pulse rate. Respiration was slightly slowed in Dogs 13 and 15; twitching and tremors were observed only in Dog 15 which had received 10 per cent. sodium chlorid solution previously. In this dog there was also an increase in sedimentation rate of the corpuscles.

As far as our results go, it would seem that 85 per cent. sucrose solution and 50 per cent. dextrose solution do not alter the blood very much, except for some dilution by both sugars and lowering of carbon dioxid by dextrose, due very likely to the dilution. After sucrose the reaction (p_H) remained practically unaltered, and after dextrose the tendency was to maintain or increase the alkalinity, though not above normal; this may be regarded as a beneficial change. Presumably, the dextrose was more readily burned to carbon dioxid than the sucrose, and this gas increased the alkali reserve though the carbon dioxid capacity did not show such an increase. There are other possibilities but without further analysis of the causes for these changes, speculation is useless. It is interesting to note, however, that as the blood dilution was recovered from (the blood concentration increased) the carbon dioxid nevertheless kept on decreasing. Perhaps twitching was more marked after sucrose, and the remaining functions that were observed were improved somewhat or not seriously impaired. It is noteworthy that the dogs that had received hypertonic solutions prior to the sugar solutions exhibited more pronounced tremors, struggling or twitching.

Urea, 18 Per Cent. Solution.—This solution was injected into two dogs of which one (Dog 16) had previously received 40 per cent. hexamethylenamin solution and one (Dog 19) 6 per cent. acacia solution. Blood dilution was marked in the dog receiving the hexamethylenamin previous to the urea solution, and only slight in the dog that had previously received acacia. The p_H values showed a tendency to decrease, though only slightly in the dog that had received acacia. The carbon dioxid and alkali reserve were increased in Dog 16 receiving hexamethylenamin and slightly increased in Dog 19, receiving acacia, before the urea. It appears, therefore, that the increase in carbon dioxid in Dog 16 may be responsible for the further increase in acidity (further decrease of p_H) while the decrease in the carbon dioxid in Dog 19

38. Macleod, J. J. R., and Fulk, M. E.: Am. J. Physiol. **42**:193 (Jan.) 1917.

may be accounted for by blood dilution, which also may account for the decreases in lactic acid and sugar. The urea content of the blood was not increased; the values remained within the normal range despite injections of large doses of the metabolite. This result agrees with the well known behavior of injected urea. The condition of shock present in Dog 16 was probably responsible for the original low p_H values of the blood in this animal. However, the injection of the hypertonic urea solution did not improve the blood pressure, although the pulse rate and respiration were temporarily improved. In fact, the collapse soon became worse, asphyxia supervened, and the carbon dioxid of the blood increased. Other symptoms in this dog were a marked fall of temperature, twitching and struggling. The sedimentation rate of corpuscles increased and hemolysis was present. In Dog 19 the symptoms were characterized by temporary increases in blood pressure and pulse rate, and by a rapid, then irregular and deepened respiration. Occasional twitching was present and the rectal temperature fell 1 degree at the end of one hour. The sedimentation rate and hemolysis of corpuscles were decidedly more marked in the blood samples after the injection of urea than in the preceding samples after acacia alone. The darkening produced by acacia was absent in the blood specimens after injection of urea. Therefore, all effects that have been described were due to the urea, the previous conditions in the dogs produced by the hexamethylenamin and the acacia being aggravated if anything by the urea. However, the effects of the 18 per cent. urea solution are not peculiar, but are in the same general direction as those of other hypertonic solutions, except for the more pronounced hemolysis, as may be expected.

The conclusion is justified that hypertonic solutions of sodium chlorid (10 per cent. solution), hexamethylenamin (40 per cent. solution) and urea (18 per cent. solution), produce definite changes in blood composition, such changes being less evident, partial or even absent after sucrose (85 per cent. solution) and dextrose (50 per cent. solution). With the sugars the chemical evidence is not as complete as would be desired. The most marked effects and symptoms were produced by hypertonic hexamethylenamin solution, and the least by the hypertonic solutions of the sugars.

HYPOTONIC SODIUM CHLORID

The effects of 0.1 per cent. sodium chlorid solution were observed in a dog (Dog 21, Table 3) that had received 100 c.c. of normal horse serum one hour before. Considerable disturbance occurred. The blood showed marked dilution, a fall of p_H, increases in carbon dioxid, lactic acid and dextrose and a practically unaltered urea. The blood pressure rose moderately and temporarily and the pulse rate was slowed; the rectal temperature fell and struggling and fascicular twitching of the

muscles were definite. Hemolysis was marked, being considerably increased over some hemolysis produced by the horse serum. When shaken the blood foamed markedly, a phenomenon not observed in other bloods of this study. The effects corresponded somewhat to those of asphyxia, although the blood was not particularly dark. The increases in carbon dioxid and lactic acid were very likely responsible for the lowering of the p_H values (acidity), in spite of blood dilution. The fascicular twitching and struggling were present when the p_H values were low. There is no doubt, therefore, that the intravenous injection of hypotonic (0.1 per cent.) sodium chlorid solution produced definite blood and symptomatic changes. This was to be expected and is a fact, in part, well known.

COLLOIDAL SOLUTIONS

All the solutions described thus far, except hexamethylenamin, have been studied previously by others with respect to many of the changes observed by us. The results obtained, therefore, serve as a check on the methods employed and further sustain the correctness of all the changes with the solutions to be described in this and the succeeding sections. The changes produced by the majority of these have not been previously reported, and certainly not with the objective constantly before us in this study. Several of the agents to be described produced anaphylactoid phenomena in guinea-pigs, and certain of them are exploited or recommended for intravenous use in human subjects. Among those relatively inert in the ordinary sense are the emulsoid colloids, whose effects are described in this section. Considerable importance is attached to the results with the colloidal agents, because despite the assumed inertness they produce definite blood changes and symptomatic effects ranging from the just demonstrable effect to death.

Agar Sol Gel, 0.1 Per Cent.—One-tenth per cent. of agar solution was boiled in 0.9 per cent. sodium chlorid solution without loss of volume until the strands disappeared. Then the mixture was filtered to remove débris. The filtrate was opaque, the agar being in the form of a gel in part, and in part in the sol state. There were no coarse aggregates. It was kept and injected warm at 38 degrees C. Such an agar mixture when injected intravenously into guinea-pigs produces violent reactions and death. The three dogs injected by us responded with marked symptoms; two of them died. The dosage of the agar in all three animals was very small, i. e., from 0.01 to 0.0125 gm. per kilogram of body weight. Apparently small dosage is not a serious drawback in the production of agar reactions. On the contrary, it appears to favor their elicitation provided the agar is finely dispersed. In Bordet's experiment with "toxified agar," for instance, the quantity of agar in the clear supernatant fluid after centrifugation is too small for

TABLE 4.—*Effects of Agar Sol Gel (0.1 Per Cent.), Acacia (6 Per Cent. and 25 Per Cent.), Dextrose (18 Per Cent.), Gelatin (5 Per Cent.) and Horse Serum*

Dog No. and Weight, Kg.	Dose per Kilogram	Time of Blood Specimen, Min.	Hemoglobin, per Cent.	CO_2 Content: p_H Colorimetric	CO_2 Content: p_H Electrometric	CO_2 Capacity: Per Cent. by Volume	Urea Nitrogen, Mg. in 100 C.c.	Lactic Acid, per Cent.	Sugar, per Cent.	Total Phosphate, Mg. in 100 C.c.	Blood Pressure, Mm. Mercury	Pulse Rate	Respiratory Rate	Rectal Temperature, C.	General Symptoms	Sedimentation Rate	Agglutination and Other Changes
Agar Sol Gel, 0.1 per Cent.																	
24 (5.4)	.....	B	106.4	7.2	....	35 36	10.9	0.1	0.25	Decreased	40	110	Art. resp. throughout	36.8	Resuscitated from collapse	—	....
	10 c.c.; preceded by shock and collapse																
	0.01 gm.	16 A	104.2	7.1	....	23 35	12.3	0.1	0.27		30	108	Some spontaneous respiration	35.4	....	+	....
		36 A	103.6	7.1	....	31 32	11.4	0.1	0.38	....	24	120	...	36.2	....	+	....
		91 A	81.0	6.8	....	28 30	11.3	0.1	0.54	Increased	10	....	...	....	Died		
25 (40)	..... 12.5 c.c.; 0.0125 gm.	B	100	7.15 (7.15, plasma)	7.11 (7.15, plasma)	....	9.3	0.09	0.13	....	80 6, left carotid 11, right carotid 10	104 104 58; 90	15 21 immediate effect Deep and rapid, 17 mins.	34.6	....	....	....
		21 A	85.5	6.95	7.04	....	....	0.06	0.11	....	16	90	3	32.4	Collapse	+	D
		26 A	77.5	7.1	7.08	....	8.9	0.06	0.11	....	10	58	Oc. gasp	....	Died	+	D
26 (11.3) No morphin	..... 10 c.c.; 0.01 gm.	B	100	7.3	7.32	48 56	9.2	0.09	0.12	5.37	120 70 increased amplitude	100 120	36 66; 72 deep	37.0	....	— 2 hrs.	....
		31 A	94.3	7.15	7.1	61 52	14.0	0.09	0.12	4.1	100	72 irregular	42	36.7	....	+ marked	....
		60 A	94.3	7.25	....	68 42	12.5	0.08	0.11	3.78	100	96 90	33 39	36.6	....	+	Blood clotting marked
	10 c.c. and later 50 c.c. 0.01 gm.	47 A	87.7	7.2	....	50 65	13.0	0.10	0.11	3.65	90	168 irregular	48	36.4	Struggling; convulsions	+	Blood very light red, nearly whitish or grey
		89 A	96.2	7.2	....	38 46	13.3	0.11	0.12	3.3	100	220	42	36.4	Struggling	+	....

Acacia, 6 per Cent.																	
								Ammonia									
18 (8.2)	..... 16 c.c.	B	100	7.0	7.08	68.7 / 76.9	14.8	+	+	....	94 50 (18 min. after injection)	66	24	37.4	....	....	....
		15 A	93.4	6.9	....	58.6 / 62.2	10.5	....	....	....	60	130	Accelerated	36.7	Twitching of paws	+	Ag
		28 A	85.5	6.9	7.0	56.8 / 60.5	10.5	+++	....	....	60	144	Accelerated	36.4	Quiet	+	Ag; D
		63 A	80.0	6.8	7.0	54.9 / 62.3	12.5	....	++	....	60	175	36	36.2	Quiet	+	H; D; Ag
19 (13.2)	..... 16 c.c.	B	100	7.2	7.14	75 / 78	8.5	+	+++	....	114 140 (5 min. after injection)	75	18	37.0	....	....	....
		32 A	97	7.1	....	73 / 78	9.3	....	++	....	120	66	18 Shallow and irregular	36.4	....	None for 8 hours + in 5 min.	H; D; Ag
		60 A	96	7.0	7.09	72 / 77	11.1	++	+	....	120	70	20 deepened	36.2	....	....	H; D; Ag
23 (8.2)	.....	B	90.1	7.1	....	48 / 49	....	0.05	....	....	84 50 (4 min. after acacia)	180	33	....	....	....	....
	16 c.c. preceded by 40 c.c. sodium iodid, 21 per cent.																
		77 A	78.4	7.0	....	28 / 36	10.5	>0.05	....	....	34	180	36 20 12 irregular and deepened	....	Collapse	+	D; Ag
		32 A	73.5	6.9	....	33 / 35	10.8	0.10	....	....	30 24	184	12 very deep 16 shallow	....	Collapse Died 5 min. later	+	H; D; Ag

TABLE 4.—*Effects of Agar Sol Gel (0.1 Per Cent.), Acacia (6 Per Cent. and 25 Per Cent.), Dextrose (18 Per Cent.), Gelatin (5 Per Cent.) and Horse Serum.—(Continued)*

Dog No. and Weight, Kg.	Dose per Kilogram	Time of Blood Specimen, Min.	Hemoglobin, per Cent.	CO_2 Content CO_2 Capacity by Volume: p_H Colorimetric	p_H Electrometric	CO_2 Capacity Per Cent.	Urea Nitrogen, Mg. in 100 C.c.	Lactic Acid, per Cent.	Sugar, per Cent.	Total Phosphate, Mg. in 100 C.c.	Blood Pressure Mm. Mercury	Pulse Rate	Respiratory Rate	Rectal Temperature, C.	General Symptoms	Sedimentation Rate	Agglutination and Other Changes
Acacia, 25 per Cent.																	
17 (6.1)	.....	B	70.4	7.2	....	48/50	15.0	+	None	....	74	192	48	36.4	....	....	....
No morphin	16 c.c. preceded by 200 c.c. sodium chlorid, 1 per cent.; 200 c.c. disodium phosphate 1.1 per cent., and 75 c.c. sodium citrate, 2 per cent.										106	156 (6 min. later)					
		9 A	.....	7.1	....	35/37	13.1	....	....	....	106	irregular	24 18 12 very deep	35.0	....	+ marked	Very D; H
		38 A	32.3	7.1+	....	34/39	13.5	....	None	....	100	Very irregular	Still deeper	....	....	+	Very D; H
	Resuscitation with sodium bicarbonate, 9 per cent., failed	53 A	30.5	6.8	6.64	52/58	17.4	++	....	....	50	15	Slowed	....	Collapse	+	Very D; H
		3 A							....	....	0	0	Stopped	....	Died	....	....
Acacia, 25 per Cent., and Dextrose, 18 per Cent.																	
20 (9.8)	.....	B	84.4	7.3	....	54/63	11.5	++	Ammonia +++	....	66	200	36	37.2	....	....	....
	5.1 c.c. preceded by 16 c.c. per kilogram gelatin, 5 per cent., and 100 c.c. disodium phosphate, 5 per cent.										120 (4 min. later)		...	....	Convulsions; continuous twitching		
		31 A	62.5	7.3	....	48/58	11.9	....	++	....	70	192	....	37.2	....	+	D
		63 A	66.6	7.25	....	46/54	12.5	+++	....	....	60	...	...	37.8	Continuous twitching	+	H; D
		78 A	66.2	7.1	....	44/55	13.3	+	+	....	50	200	...	37.6	Twitching less	+	H; D

Gelatin, 5 per Cent.

								Ammonia								
20 (9.8)	.....	B	100	7.1	6.96	67/68	11.6	+	+	....	130	80	20	37.6	....	....	....
	16 c.c.	30 A	86.9	6.9	7.0	59/66	12.5	....	++	....	120	126	24	37.2	Struggling; irregular twitching of muscles	+ marked	D
		62 A	99.0	6.9	....	57/65	13.8	++	....	....	120	166	24	36.6	....	+	D
		87 A	89.3	6.9	6.97	56/60	....	+++	....	....	90	...	...	....	Twitching more marked; increased reflex excitability	+	....
								Horse Serum								
								Ammonia								
21 (6.1)	.....	B	85.1	7.0	....	52/57	12.5	+	+	....	20 46 (7 min. later)	94	...	31.8	....	....	H slight
	16 c.c. preceded by 200 c.c. Tyrode's solution	30 A	77.8	7.3	....	52/57	13.0	....	+	....	44	...	..	....	....	None	H slight
		60 A	77.8	7.35	....	52/57	11.1	++	++	....	44	60	...	29 8	....	None	H marked

detection, yet such a serum produces a most violent reaction and death in the same unsensitized animal from which the serum was obtained for incubation with the agar. The serum so treated by agar is not altered chemically and contains no demonstrable toxic products. However, it appears from the studies of several investigators that some change in its physical state and the minute but undetectable quantity of dissolved agar, which may occur, are the determining factors in its toxicity. Similar changes and effects are produced after treatment of serum with starch, insulin and kaolin and perhaps with other agents. Serum is not indispensable in the "toxified agar" mixture, since agar alone when properly treated so as to give the form of the sol gel described above elicits the same reactions, at least in guinea-pigs. We may now describe the toxic effects of agar sol gel in the dogs.

The results in Table 4 were obtained on one morphinized dog in a state of shock, and on two others in good condition, one of whom (Dog 26) was not morphinized. Fatal effects were produced in Dog 24 in shock and in Dog 25 in good condition at the beginning. Marked effects were produced in Dog 26, morphinized and in good condition. Shock may have been a contributory factor to the sudden death of Dog 24, but it certainly was not of Dog 25 which also died suddenly after the injection. Hence, it is apparent that it was the agar that was the determining factor in bringing on sudden death in both dogs. The tracing in Figure 1 illustrates the profound circulatory and respiratory changes occurring in Dog 25 and typifies one kind of reaction to agar. Another kind of reaction is illustrated by Figure 2, which shows several portions of a continuous tracing obtained over two and one-half hours from Dog 26.

The agar injection in this dog was not fatal within the time limit of the experiment, but nevertheless caused very marked changes in the circulation and respiration. That is, the blood pressure fell considerably immediately after the injection; then recovered to the previous level for a short time, and later fell progressively with occasional sharp falls and partial recoveries, the level reached at the end of two and one-half hours being decidedly lower than the initial level. The changes in pulse were even more marked, being characterized by slowing with a very marked increase in amplitude and some irregularity for about an hour after the injection. After that there was a recovery in amplitude and an increase in rate. The respiratory changes were characterized by irregularity in rate and amplitude. Struggling and convulsions were present during the time of circulatory collapse.

The tracing in Figure 1, which typifies the more marked reaction in the other two dogs, was obtained from Dog 25 when in good condition. It is self explanatory, and leaves no doubt that profound effects are

possible from small doses of agar. The details pertaining to the blood pressure changes and respiratory rates in all dogs are given in Table 4. Two minutes after the injection, the blood pressure from the left carotid artery fell to the zero level and showed no signs of recovery at the end of twenty minutes. After satisfying ourselves that the recording system

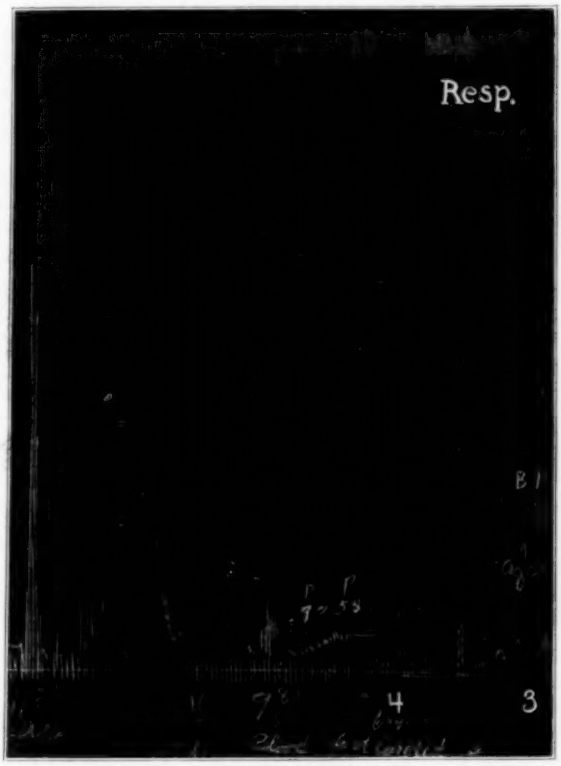

Fig. 1.—Effects of 0.1 per cent. agar sol gel (0.011 gm. per kilogram) intravenously in Dog 25, which weighed 4.4 kg. *P*, pulse, *R*, respiration; at 3, blood pressure from left carotid artery; at 4, blood pressure from right carotid artery. Blood samples were taken at 8 and 9. Resuscitation began at 10, aqueous fluid extract of mistletoe, 0.5 c.c. per kilogram, being administered intravenously; at 11, the same dose of mistletoe was given and in addition cardiac massage, artificial respiration and intravenous injection of 0.9 per cent. sodium chlorid, but the resuscitation was unsuccessful. Time: each stroke equals twenty seconds.

attached to this carotid artery was capable of transmitting changes, the attachments were switched to the right carotid artery, which revealed a blood pressure of only 16 mm.; five minutes later this was reduced to the zero level. This showed that the left carotid artery had been record-

ing the pressure changes faithfully. Resuscitation with mistletoe, cardiac massage and artificial respiration were tried but gave only temporary recovery, and the dog died. The respiration lasted about as long as the heart continued to beat, and was very irregular in both rate and amplitude. The changes in Dog 24 were essentially the same though more lasting. Dogs 24 and 25 died without recognizable struggling, tremors or convulsions.

As to the physical and chemical changes in the blood, these were definite and rather marked in all the dogs. Dilution was considerable; the reaction tended toward acidity (lowered p_H); carbon dioxid was reduced, and this reduction was greater than the blood dilution would

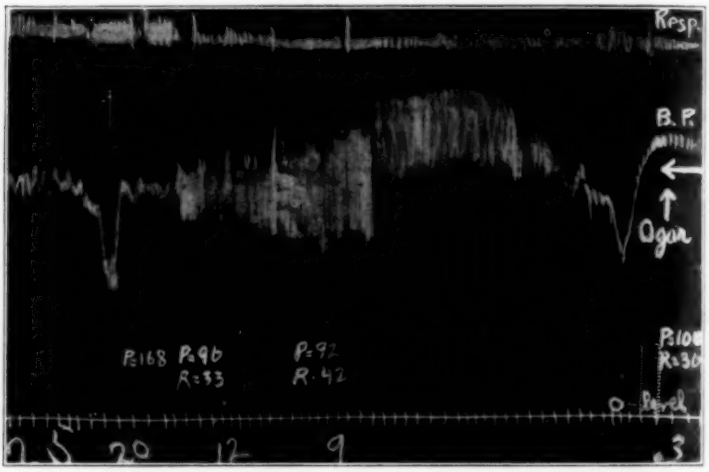

Fig. 2.—Effects of 0.1 per cent. of agar sol gel (0.01 gm. per kilogram) intravenously in Dog 26, which weighed 11.3 kg. At 3, injection of agar sol gel with effects during the next eight minutes; at 9, end of thirty-one minutes; at 12, end of fifty-three minutes; at 20, end of one hour and forty-five minutes, and at 25, end of two hours and twenty-nine minutes. Each stroke in record equals twenty seconds. The figures on the record correspond to withdrawal of successive samples of blood; the details pertaining to the respiratory and pulse rates, blood pressure and blood changes accompanying these are presented in Table 4.

indicate. The urea-nitrogen and lactic acid tended to increase in two (Dogs 24 and 26) and were lowered in one (Dog 25), while the phosphate tended to decrease in the two dogs that were tested for this. Perhaps the reason that the changes in Dog 25 do not appear more marked, or even appear opposite to those in the others, is that the animal suffered very rapid collapse and did not live long enough. The sedimentation rate of the bloods from all the dogs increased after the agar injections, and agglutination was present in all. Darkening was

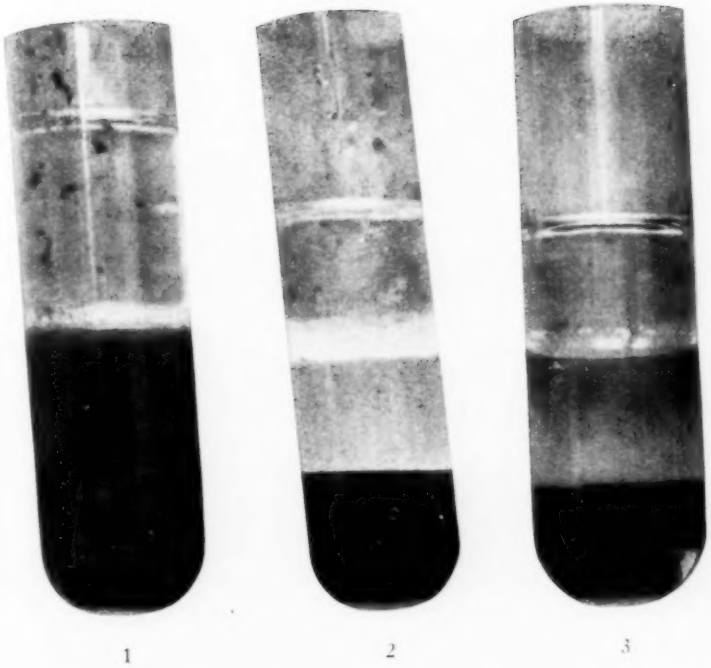

Fig. 3.—Physical changes in arterial blood of Dog 23 after intravenous injection of 6 per cent. solution of acacia, and 21 per cent. solution of sodium iodid. The blood in all cases was collected under liquid petrolatum. In Tube 1, the control, no changes took place; in Tube 2, 21 per cent. solution of sodium iodid, there was rapid sedimentation and darkening of corpuscles, and the plasma was clear fifteen minutes after the injection of sodium iodid; in Tube 3, 6 per cent solution of acacia, there was rapid sedimentation and darkening of corpuscles, hemolysis and agglutination of corpuscles in the plasma layer thirty minutes after the acacia was given. The clear uppermost layer in each tube is liquid petrolatum.

pronounced in Dog 25. Clotting was rapid in Dog 26 and the blood appeared grayish. Everything together, therefore, indicates that, despite its inertness in the ordinary sense, the intravenous injection of small doses of agar causes profound symptomatic changes and even death and definite changes in the composition and appearance of blood.

Acacia, 6 and 25 Per Cent. Solution.—Acacia has been used in both these concentrations in human subjects, although to a greater extent in the 6 per cent. concentration. Its advocates have denied the possibility of deleterious effects from its use, but our results on dogs sustain those who condemn its use. The results after 6 per cent. acacia solution were uncomplicated by other injections in two dogs, and only by iodid in one dog; those after 25 per cent. acacia solution were complicated by several other injections, although the changes following acacia agree with those of uncomplicated injections.

Taking first the results with 6 per cent. acacia solution in 0.9 per cent. sodium chlorid solution (Table 4), it is seen that there was considerable blood dilution in Dogs 18 and 23, a tendency toward acidity (lowered p_H) and a reduction in carbon dioxid. The latter was accounted for partly by the blood dilution. Lactic acid was increased in the bloods of all the animals and probably contributed, therefore, to the reduction in blood alkalinity. Phosphate was not estimated; urea tended to fall, and the ammonia was variable. Hemolysis, increased sedimentation rate, agglutination and darkening were present in the bloods of the three dogs. These changes were rather striking, and are well illustrated by Tube 3 in Figure 3, reproduced from a Lumière autochrome plate. The increased sedimentation rate can be partly explained by the blood dilution, but dilution alone cannot explain hemolysis, agglutination and darkening. The latter changes arise from the acacia. Agglutination, which sometimes is accompanied by hemolysis, can be reproduced by contact of the drug with washed corpuscles in vitro, as has been shown by Karsner and Hanzlik.[1] In this laboratory a rather marked darkening and peculiarly coarse appearance of the blood has frequently been observed by different workers using the acacia solution for resuscitation or for maintaining high blood pressure during experimentation on animals. When such changes supervene the animals are apt to die suddenly or very soon after injection.

Symptoms of twitching in the paws were present in Dog 18; in Dog 23 collapse was followed by death thirty-two minutes after injection. The blood pressure fell in Dogs 18 and 23 and was only moderately raised in Dog 19; pulse rates and respiratory rates were variable. Irregularity of the respiration was particularly marked. The rectal temperature fell as usual in morphinized animals. It can certainly be said that 6 per cent. acacia solution did not act beneficially in Dog 23

with a moderately reduced blood pressure, a condition that should have been improved, according to the advocates of the solution. The effect was the contrary and the animal died, though it is true that it had received 21 per cent. iodid solution (the same as is used intravenously for therapeutic purposes in patients) fifty-one minutes prior to the acacia. The effects on blood pressure were no better, of course, in Dog 18 which had not received prior intravenous injections of any kind and whose initial blood pressure was 94 mm. This indicated that it was not the iodid per se in Dog 23 that led to the fatal result but, rather, that the acacia was to be blamed.

Such results do not inspire confidence in 6 per cent. acacia solution as a corrective for circulatory depression and collapse. Our experiences with acacia are in line with the unfavorable reports of Henderson and Haggard,[39] who compared it with other resuscitating solutions, of Richet, Brodin and Saint-Girons,[40] who tried it in hemorrhage, and of Meyer,[41] who prefers modifications of Locke's or Ringer's solutions. According to Hammarsten,[42] gum inhibits or prevents the clotting of blood, and delay in blood coagulation of dogs after acacia solutions has been reported by Foster and Whipple,[43] who also found increases in the nonprotein nitrogen. Nonnenbruch [44] observed increases of 100 per cent. in serum protein of rabbits that had received acacia. The significance of these nitrogen and protein changes is not known, but they are at least in line with a fact hitherto insufficiently appreciated, namely, that blood is changed by intravenous injection of acacia.

Our results with 25 per cent. acacia solution are complicated by those of other injections and we do not wish to overestimate them. However, we submit them for the value which they do possess. Both dogs (Dog 17, which received 25 per cent. acacia solution alone, and Dog 20, which received 25 per cent. acacia solution containing 18 per cent. dextrose solution) had low levels of blood pressure, 74 and 66 mm. of mercury, respectively; that is, they were in a condition in which the acacia solutions were indicated. Yet the ultimate results were not beneficial, since death occurred in Dog 17 and collapse became more pronounced in Dog 20, despite the fact that the blood pressures in both dogs were temporarily improved. As for the other changes, these were precisely the same as those after injections of 6 per cent. acacia solution. Figure 4

39. Henderson, Yandell; and Haggard, H. W.: Hemorrhage as a Form of Asphyxia, J. A. M. A. **78**:697 (March 11) 1922.
40. Richet, C.; Brodin, P., and Saint-Girons, F.: Presse méd. **26**:581 (Nov. 14) 1918.
41. Meyer, Erich: Klin. Wchnschr. **1**:1 (Jan. 1) 1922.
42. Hammersten-Mandel: Textbook of Physiological Chemistry, Ed. 7, p. 312, 1914.
43. Foster, D. P., and Whipple, G. H.: Am. J. Physiol. **58**:393 (Jan.) 1922.
44. Nonnenbruch: Arch. f. exper. Path. u. Pharmakol. **91**:218, 1921.

illustrates the circulatory and respiratory changes and Table 4 contains the details. They need not be repeated here. However, we wish to point to the rather marked decreases in p_H, i. e., tendency toward acidity of the blood, despite previous injections of alkaline phosphate and citrate in Dog 17 and of phosphate in Dog 20. Increased sedimentation rate, hemolysis and darkening of the bloods were present in both dogs, the darkening suggesting an asphyxial state which supervened in both dogs spontaneously after injection of the acacia solutions. Thus it is seen that, under the conditions, the changes produced by the 25 per cent. acacia solutions were essentially the same as those produced by 6 per cent. acacia solution alone. Increases in plasma protein, sugar, chlorid, urea and nonprotein nitrogen of the blood after injection of hypertonic acacia solution and glucose into normal, asphyxiated and shocked dogs have been reported by White and Erlanger.[45] Hemolysis also was

Fig. 4.—Effects of 25 per cent acacia solution in 0.9 per cent. sodium chlorid (16 c.c. per kilogram) intravenously in Dog 17, which weighed 6.1 kg. *P*, pulse; *R*, respiration. Blood samples were taken at 14, 15 and 16. Each stroke in the record equals twenty seconds.

observed by these authors. They state that this was no greater than that of the controls, and that they consider the claims of Kruse [46] misleading. Our results agree with the report of Kruse. As for injurious effects from acacia solutions of different concentration and composition, the work of Erlanger and Gasser [47] indicates that the solutions as a rule did not reduce mortality but that, on the contrary, certain mixtures of acacia increased the mortality of dogs in different conditions, all of which indicates that claims of the harmlessness of injected acacia cannot be made without reservation.

45. White, H. L., and Erlanger, J.: Am. J. Physiol. **54**:1 (Nov.) 1920.
46. Kruse: Am. J. Physiol. **49**:137, 1919.
47. Erlanger, J., and Gasser, H. S.: Am. J. Physiol. **50**:119, 149 (Oct.) 1919; Ann. Surg. **69**:389 (April) 1919.

Gelatin, 5 Per Cent. Solution.—This was tried in one dog without preceding injections. The results in Table 4 show that the changes produced were essentially the same as those from acacia. The symptoms were somewhat more marked: muscular twitching was more marked; there was increased reflex excitability, and the blood pressure fell progressively, while the pulse and respiratory rates were augmented. An increased sedimentation rate and darkening of the blood were definite; dilution was marked and lasting, and there were progressive increases in lactic acid, ammonia and urea with reduction in carbon dioxid and a tendency to increase in hydrion concentration (lowered p_H). The reduction in carbon dioxid cannot be altogether accounted for by the blood dilution, and it appears that the increase in lactic acid contributed to the reduction in blood alkalinity (p_H).

Horse Serum.—Ordinary normal horse serum preserved with 0.3 per cent. tricresol was injected in the dosage of 16 c.c. per kilogram into Dog 21, which had previously received 200 c.c. of Tyrode's solution. The results (Table 4) show that, with the exception of less than 10 per cent. of blood dilution and moderate increases in lactic acid and ammonia and a slight increase in hemolysis, the net result was beneficial. That is, alkalinity of the blood was increased to normal (p_H 7.35); the carbon dioxid remained unchanged, and the blood pressure was raised over 100 per cent., having been at the extreme low level of 20 mm. of mercury before injection of the serum. The pulse rate was slowed and the temperature fell, though no more than ordinarily occurs in morphinized animals. The urea changes were unimportant. The increase in lactic acid here was seen to occur during an increase in alkalinity of the blood, which is the opposite tendency to the acidity accompanying the injections thus far described. However, the occurrence of an increase in lactic acid during an increase in blood alkalinity is known to occur under other circumstances. Anrep and Cannan [48] have shown that the lactic acid in the blood rises when the alkalinity of the blood increases and that a fall in alkalinity is followed by a decrease of lactic acid in heart-lung preparations and spinal animals. Macleod and Knapp [49] have demonstrated an increase in lactic acid in the blood after administration of sodium bicarbonate to rabbits, and it will be seen later on in this paper that we have confirmed the increase after bicarbonate injections in dogs. This simply means that increases in lactic acid can occur under different conditions and, according to Himwich, Loebel and Barr [50] and Liljestrand and Wilson,[51] an increase normally occurs during exercise. The

48. Anrep, G. V., and Cannan, R. K.: J. Physiol. **58**:244 (Dec.) 1923.
49. Macleod, J. J. R., and Knapp, A.: Am. J. Physiol. **47**:189 (Nov.) 1918.
50. Himwich, H. E.; Loebel, R. O., and Barr, D. P.: J. Biol. Chem. **59**:265 (March) 1924.
51 Liljestrand and Wilson: Proc Soc. Exper. Biol. 21:426, 1924.

increases during alkalinity and acidity signify alterations in cellular functions; whether these are in the musculature or in the tissues as a whole or as a result of anoxemia or asphyxial states is immaterial to the present study. For, we are primarily concerned with the demonstration of the facts of the disturbances in the physical and chemical mechanisms which, it is believed, are fundamentally concerned in the functional alterations of cells. In the case of horse serum, the minor disturbances that followed its injection apparently were not detrimental. In fact, the results as a whole indicate, if anything, an improvement in the dog's condition. The results agree in general with what is known of the relatively low toxicity of horse serum. It appears that a nontoxic serum would be a more suitable medium or vehicle for intravenous injections than such foreign agents as acacia, agar and the others to be described. For this reason, blood transfusion is to be preferred to acacia, balanced and isotonic solutions and other analogous artificial substitutes in the treatment of circulatory depression, collapse and shock.

SUSPENSIONS

The following suspensions of insoluble and particulate materials were tried: barium sulphate, 3 per cent., and fullers' earth, 0.5 per cent., both in 0.9 per cent. sodium chlorid solution. The results with barium sulphate were uncomplicated and rather unexpected, while those with fullers' earth were complicated with those of previous injections. The results are presented in Table 5.

Barium Sulphate, 3 Per Cent. Suspension.—The intravenous injection of freshly prepared barium sulphate in a more or less gelatinous and amorphous state in guinea-pigs and pigeons causes violent reactions and death. Lumière reports similar effects in dogs after intracarotid injection toward the brain; according to him, the reactions are not elicited when the barium sulphate is in a coarsely granular state. The barium sulphate used by us was prepared according to Lumière's [52] method, and the dosage used was the same as that used by Lumière, namely, 0.019 gm. per kilogram. Three injections were made into the saphenous veins of two dogs and one injection was made into the carotid, after the manner of Lumière. Besides the usual analyses of arterial blood from the femoral artery, p_H and hemoglobin estimations in the venous blood from the right ventricle (by chest puncture) also were made. This was done in anticipation of some disparity in the circulation of the venous and arterial sides as a result of the particulate injection.

The results in both dogs were uniform. Certain changes were decidedly interesting, though negligible as far as the p_H, urea, lactic acid, sugar and phosphate are concerned. The important blood changes pertained to

52. Lumière, A.: Le Probleme de L'Anaphylaxie, Paris, p. 241, 1924.

TABLE 5.—*Effects of Suspensions of Barium Sulphate, 3 Per Cent., and of Fullers' Earth, 0.5 Per Cent.*

Dog No. and Weight, Kg.	Dose per Kilogram	Time of Blood Specimen, Min.	Hemoglobin, per Cent.	p_H Colorimetric	p_H Electrometric	CO_2 Content / CO_2 Capacity Per Cent. by Volume	Urea Nitrogen, Mg. in 100 C.c.	Lactic Acid, per Cent.	Sugar, per Cent.	Total Phosphate, Mg. in 100 C.c.	Blood Pressure, Mm. Mercury	Pulse Rate	Respiratory Rate	Rectal Temperature, C.	General Symptoms	Sedimentation Rate	Agglutination and Other Changes
Barium Sulphate, 3 per Cent.																	
29 (16) No morphin	.....	B	100	7.3	....	58/61	12.5	0.23	0.13	5.13	114	84 60 96	24 18	37.0	....	....	....
	0.63 c.c.; 0.019 gm.	36 A	104.2	7.35	....	62/64	13.3	0.21	0.14	4.27	100	114 70 Irregular; large pulse volume	... 18 Irregular	....	Twitching	+	H; Ag; D
	0.63 c.c.; 0.019 gm.	41 A*	87.3	7.25													
		39 A	95.7	7.5	....	59/61	13.3	0.22	0.13	4.55	100	96 78 80	18 18 Irregular	36.6 36.7	Tremors; twitching	.... +	.... More H; Ag; D
		36 A*	93.4	7.2					....	....	....	84 Irregular	20 Irregular	....	....	....	still more H; Ag; D
32 (18.4) ½ dose	.....	B	100	7.25	....	48/50	11.4	0.26	0.07	2.91	140	100 102	84 72	36.8	....	+	....
	0.54 c.c.; 0.016 gm. intravenously										120	...	...	....	Tremors	....	....
		10 A*	106.9	7.25	....	..	....	....	....	....	126	108	90	36.8	....	++	H; Ag
		30 A	114.2	7.25	....	50/52	11.7	0.38	0.07	2.5							
		32 A*	105.3	7.25	....	..	....	....	....	....	120	120	30 deep	36.8	....	....	....
	0.54 c.c.; 0.016 gm. intracarotidially										130 (before) 150 (5 min. after barium)						
		37 A	113.6	7.30	....	52/53	12.1	....	....	2.7	136	156	78	36.85	2 convulsions	++	H slight
		39 A*	110.5	7.25	....	..	....	0.27	0.07	....	134	108	108	37.2	....	....	....
Fullers' Earth, 0.5 per Cent.																	
29 (16) No morphin	.....	B	95.7	7.5	....	59/61	13.3	0.22	0.13	4.55	100	84	20	36.7	Twitching	....	H; Ag; D
	6.3 c.c. preceded by 20 c.c. barium sulphate, 3 per cent. 0.052 gm.										42 (2 min. after injection)						
		10 A*	84.7	7.35	....	53/58	14.6	0.21	0.12	3.79	10	138	30 deep	36.6	....	....	H; Ag; D
		16 A†	93.0	7.35	....	..	15.0	0.47	0.11	3.74	0	168 Irregular	54 deep and irregular	....	Died	Blood granular	H; Ag; D
28 (5.5)	.....	B	....	....	....	..	....	....	....	....	40	40	8	....	....	....	....
	12.4 c.c. preceded by arsphenamin, 5 c.c. of 0.35 per cent.	4 A	....	....	....	..	....	....	....	....	0 (resuscitation failed)	0	10	....	....	....	....

* Blood from right ventricle.

the hemoglobin, the carbon dioxid and the physical appearance. At the end of about half an hour after injection, the arterial bloods of both dogs showed increases in hemoglobin and carbon dioxid without significant changes in p_H. A second injection in Dog 29 half an hour later again raised the hemoglobin as compared with the preceding value, but the carbon dioxid remained practically unchanged as compared with the original control values and the blood became more alkaline (p_H 7.5). The changes in hemoglobin indicate increased blood concentration, which would explain the increase in carbon dioxid in part at least. The venous blood from the right ventricle of Dog 29 tended to have lower hemoglobin and p_H values than the arterial blood. The same difference existed between the ventricular venous and femoral arterial bloods of Dog 32 as far as hemoglobin was concerned but not with respect to the p_H. The bloods of both dogs showed an increased sedimentation rate, hemolysis and agglutination, and there was darkening in Dog 29. The symptoms were characterized by twitching and tremors, a moderate fall of blood pressure, temporary acceleration of the pulse, variable respiration, a slight fall of temperature in Dog 29 and an increase in Dog 32.

The intracarotid injection in Dog 32 caused two convulsions with marked increases in blood pressure, pulse and respiration and 0.4 degree C. rise in temperature. The arterial blood remained concentrated from the previous intravenous injection; the carbon dioxid increased still more, and the p_H value only slightly (alkaline tendency). The p_H and hemoglobin values of the venous blood were smaller than of the arterial blood. The interpretation of the results with barium sulphate is difficult. Some disparity in the circulation of blood between the arterial and venous sides appears to have occurred, as indicated by the hemoglobin changes. However this may be, the chemical and physical changes in the blood were not pronounced and the symptoms, though definite, were only moderate. All the changes were less marked than those with similar doses of agar and some other agents described in this article. The volume of the barium sulphate suspension injected was less. However, it is not believed that this difference would explain the difference in behavior of dogs toward agar and barium sulphate. Rather it appears that a substance in order to be reactive must be in a soluble or highly dispersed state, as is more apt to be the case with crystalloids and emulsoids than with a coarse, insoluble precipitate like barium sulphate. It has been observed by one of us (F. D.) that pigeons do not react to old, standing or aggregated barium sulphate, but do so violently to the freshly precipitated and gelatinous barium sulphate. The difference is striking and is concerned solely with a difference in dispersion of the precipitate. That is, the reaction is more marked as the dispersion increases.

Fullers' Earth, 0.5 Per Cent. Suspension.—A dose of 0.032 gm. per kilogram was injected into Dog 29, which had received barium sulphate previously. The injection was promptly fatal. The blood pressure fell precipitously from 100 to 10 mm. of mercury, the pulse and respiration became greatly accelerated, and presently both stopped (end of sixteen minutes). The changes in these functions are illustrated by Figure 5. There was some dilution, a tendency to acidity (lowered p_H), a decrease in carbon dioxid and phosphate, a slight fall in sugar, and increases in urea and lactic acid of the blood. Essentially the same symptomatic changes and also prompt death occurred in Dog 28, which received double the dose of fullers' earth after an injection of arsphenamin from

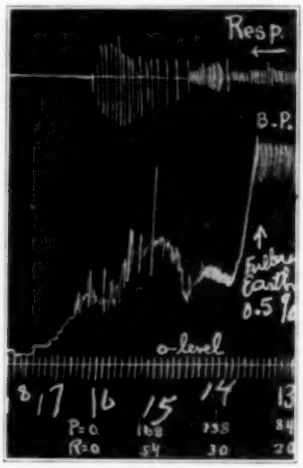

Fig. 5.—Effects of 0.5 per cent. suspension of fullers' earth (0.032 gm. per kilogram) intravenously in Dog 29, which weighed 16 kg. At 14, twitching; at 17 and 18, blood samples were taken from right and left ventricles, respectively. *P*, pulse; *R*, respiration. Each stroke of the record equals twenty seconds.

which it had partly recovered. Resuscitation was fruitless and death occurred so rapidly that no blood for analyses could be secured. From these results there is no doubt that small doses of fullers' earth are promptly fatal to dogs. When the agent is highly dispersed, it is also fatal to guinea-pigs, the effects being largely physical.

MISCELLANEOUS AGENTS

Among miscellaneous agents are included a number of salts and drugs used intravenously in human subjects and certain agents that produce violent anaphylactoid reactions in guinea-pigs. The results with all the agents in this section are presented in Table 6.

Arsphenamin.—Disodium arsphenamin was prepared in the usual manner for human subjects, using the upper limit of therapeutic dosage, namely, 0.6 gm. This made the dosage 0.015 gm. per kilogram for the two dogs that were observed. In one (Dog 27) the injection was uncomplicated, but in the other (Dog 28) it was preceded by peptone. However, the changes following arsphenamin were essentially the same, except for some differences in blood dilution and carbon dioxid. In the dog receiving arsphenamin alone, there was a steady increase in blood dilution to the end of 105 minutes, while in the dog that received peptone blood dilution was absent; in fact, there was some increase in concentration at the end of sixty-five minutes, as though the capillaries that had been acted on by the peptone in this dog allowed better escape of the injected solution from the circulation. In the dog that received peptone, there was a steady and considerable decrease in the carbon dioxid despite the unaltered concentration of the blood, while in Dog 27,

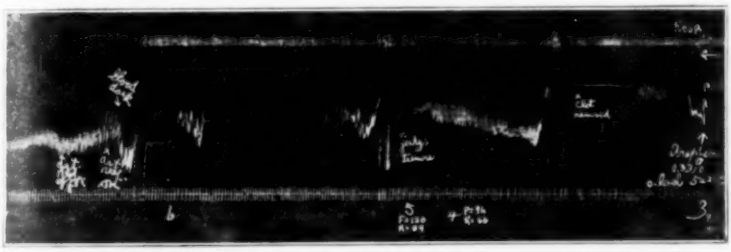

Fig. 6.—Effects of arsphenamin (0.017 gm. per kilogram) intravenously in Dog 27, which weighed 6.7 kg. *P*, pulse; *R*, respiration. Blood samples were taken at 5, 6 and 7. Each stroke of the record equals twenty seconds.

which received arsphenamin alone, the carbon dioxid remained practically unchanged during one hour after administration of the drug, and only fell at the end of 105 minutes, when the blood became decidedly more diluted. With the exception of a considerable increase in p_H (7.5) at the time of some blood concentration in the dog that received peptone and arsphenamin, the p_H in both dogs at all other times fell definitely. At no time, however, did it reach neutrality or acidity, perhaps because to start with the bloods were quite alkaline. When it is borne in mind that disodium arsphenamin is itself strongly alkaline, the fall in the p_H values as a result of the injections was really considerable. Another marked effect was on the lactic acid content, which steadily increased in both dogs despite variations in blood concentration, and reached increases of about 100 per cent. in both dogs at the end of one and one-half hours. Presumably this increase contributed to the reduction of the p_H. Whether the alkalinity of the disodium arsphen-

TABLE 6.—*Effects of Miscellaneous Agents*

Dog No. and Weight, Kg.	Dose per Kilogram	Time of Blood Specimen, Min.	Hemoglobin, per Cent.	p_H Colorimetric	p_H Electrometric	CO_2 Content / CO_2 Capacity Per Cent. by Volume	Urea Nitrogen, Mg. in 100 C.c.	Lactic Acid, per Cent.	Sugar, per Cent.	Total Phosphate, Mg. in 100 C.c.	Blood Pressure, Mm. Mercury	Pulse Rate	Respiratory Rate	Rectal Temperature, C.	General Symptoms	Sedimentation Rate	Agglutination and Other Changes
Arsphenamin, 0.33 per Cent.																	
27 (6.7) ½ dose	..... 5 c.c.; 0.015 gm.	B	100	7.6	7.63	44/47	9.6	0.065	0.094	6.45 (2 min. later	90 74	96 120 96	84 72 66	37.4 36.8) 36.2	....	....	....
		31 A	96.2	7.3	7.36	45/48	10.0	0.092	0.119	6.18	70	120	84 jerky; irregular	36.0	Tremors	+	H; Ag
		60 A	92.2	7.3	7.37	45/48	9.5	0.116	0.14	5.73	70	...	Art. resp.	....	....	+	H; D; Ag marked
		105 A	88.5	7.2	7.31	41/45	10.2	0.102	0.168	5.91	44	...	Art. resp.	....	....	....	H; D; Ag
28 (5.5) ½ dose	..... 5 c.c.; 0.015 gm.	B	101.5	7.3	7.33	51/56	....	0.43	0.16	4.54	70	144 180	24 30	35.2 35.2	....	+	Ag
		preceded by 5 per cent. peptone, 0.38 c.c. per kilogram															
		33 A	100.0	7.2	7.25	46/50	....	0.59	0.16	4.65	78	144	30	....	....	+ more marked	H; Ag
		65 A	109.3	7.5	7.51	45/49	....	0.91	0.18	4.48	80	144	30 irregular	....	....	+ still more marked	H; Ag
		85 A	99.5	7.2	7.25	43/45	....	0.91	0.19	4.81	40 accidental	...	...	....	....	+	H; D; Ag
Calcium Chlorid, 9 per Cent.																	
18 (8.2)	..... 5.5 c.c.; 0.5 gm.	B	98.5	6.9	7.0	51.2/54.9	13.3	++	+	....	80	158	30	35.2	....	+	H
		preceded by 6 per cent acacia, 16 c.c. per kilogram and 10 per cent. sodium chlorid, 6.1 c.c. per kilogram															
		24 A	67.7	6.8 or 7.0	6.74	42.1/49.5	14.8	+	+	....	30	96 0	30 Art. resp.	....	Died	None	H
19 (13.2) ½ dose	..... 5 c.c.; 0.45 gm.	B	79.0	7.4	7.27	140/140	11.5	++	+ tr.	....	134	87	27	34.6	....	+	H
		preceded by 6 per cent. acacia, 16 c.c., 18 per cent. urea, 7.6 and sodium bicarbonate, 15 c.c. per kilogram															
		35 A	....	7.4	7.26	96/97	11.1	....	....	....	134	48	24	34.6	....	+ slow	H
		60 A	90.5	7.4	7.25	96/98	11.7	+	+ (?)	....	130	...	...	....	Killed	None	H

Copper Sulphate, 5 per Cent.																	
30 (10.2) ½ dose	.....	B†	100	7.25	....	61/65	13.2	0.146	0.107	6.18	120	120	24	36.8	....	....	....
		B*	103.6	7.25	....	58/—	....	.....	....	....	...	108	36 after 1 min.	....	2 convulsions	....	....
	0.98 c.c.; 0.049 gm.									(after 3 min., 70)			Deep; shallow; stopped				
		11 A	121.3	7.1	....	50/50	10.8	0.118	0.086	6.33	40	54	48 deep	36.6	Continuous, moderate convulsions; salivation	+	H
		13 A*	112.4	7.1	....	55/57	....	....	....	....	16	0	Oc. gasp	....	Died	....	H; D; no blood coagulation end of ½ hour
32 (18.4) ½ dose	.....	8†	113.6	7.3	....	52/53	12.1	....	....	2.71	134	168	108	37.2	....	....	....
		B*	110.5	7.25	....	..	....	0.27	0.065	....	...	...	...	....	....	+	H
	0.54 c.c.; 0.027 gm.	preceded by 10 c.c. barium sulphate, 3 per cent., intravenously and 10 c.c. intracarotidly									30 2 min. later		Deep and accelerated				
		7 A†	105.3	7.15	....	34.4/36.7	10.9	0.21	0.054	+ too low to estimate	0	54	...	Oc. gasp	Convulsions	....	....
		9 A*	105.8	7.2	....	....	....	....	....	....	0	0	0	....	Died	+	H; Ag more; no blood coagulation end of ½ hour
Histamin, 0.01 per Cent.																	
26 (11.3) No morphin	.....	1 B	96.2	7.2	....	38/46	13.3	0.114	0.124	3.3	106	220	42	36.4	....	+ marked	....
	0.68 c.c. in five divided doses; 0.000068 gm., preceded by 20 c.c. agar, 0.1 per cent.																
		23 A	96.2	7.15	....	46/40	12.0	0.096	0.106	3.38	60	180	Irregular	....	....	+ less marked	....
	0.31 c.c. in three divided doses; 0.000031 gm.																
		14 A	....	7.2	....	29/59	13.4	0.133	0.106	3.50	46	...	Irregular; accelerated	....	....	+ most marked	....
	0.4 c.c.; 0.00004 gm.																
		22 A	97.1	7.15	....	26/34	12.4	0.09	0.106	3.80	46	180	60 Deepened	....	....	+ less marked	....
		35 A	98.0	7.2	....	28/35	12.4	0.078	0.100	4.07	80	...	Deepened and slowed	....	....	+ most marked	H

TABLE 6.—*Effects of Miscellaneous Agents.—(Continued)*

Dog No. and Weight, Kg.	Dose per Kilogram	Time of Blood Specimen, Min.	Hemoglobin, per Cent.	p_H Colorimetric	p_H Electrometric	CO_2 Content / CO_2 Capacity Per Cent. by Volume	Urea Nitrogen, Mg. in 100 C.c.	Lactic Acid, per Cent.	Sugar, per Cent.	Total Phosphate, Mg. in 100 C.c.	Blood Pressure, Mm. Mercury	Pulse Rate	Respiratory Rate	Rectal Temperature, C.	General Symptoms	Sedimentation Rate	Agglutination and Other Changes
						Peptone, 5 per Cent.											
27 (6.7) No morphin	.....	B	88.5	7.2	7.31	41/45	10.2	0.102	0.168	5.91	34	120 132 144	84 24 24	36.0	....	+	H
	0.38 c.c.; 0.019 gm.	preceded by 0.33 per cent. arsphenamin, 5 c.c. per kilogram															
		33 A	89.3	7.06	7.05	31/37	10.5	0.180	0.156	6.32	30	126	22	....	....	+	H; Ag
		60 A	82.3	7.1	7.15	25/30	11.8	0.105	0.118	8.29	26	0	Slowed	Collapse; resuscitation	....	+ most marked	H; Ag
28 (5.5) ½ dose	.....	B	100	7.3	7.28	42/47	....	0.06	0.18	5.07 (0 min. later	90 20	108 156	40 42) deep	36.0	....	....	....
	0.38 c.c.; 0.019 gm.	30 A	105.3	7.2	7.21	52/53	....	0.53	0.15	4.89	90	104 144	... 24 shallow	35.2 35.2	....	+	Ag
		62 A	101.5	7.3	7.32	51/56	....	0.43	0.16	4.65	90	...	Irregular	....	....	+ more	Ag
						Sodium Bicarbonate, 9 per Cent.											
16 (5.1) No morphin	.....	B	51.1	6.6	6.59	27/30	12.9	....	....	....	14	48	Art. resp.	....	....	Marked +	H
	9.8 c.c.; 0.88 gm.	preceded by 50 c.c. hexamethylenamin, 40 per cent., and 50 c.c. urea, 18 per cent.										78	Art. resp	31.6			
		11 A	42.6	7.3+	7.2	92/86	19.0	....	....	....	30	84	Art. resp.	....	....	Less +	H
		30 A	52.1	7.15	7.02	66/63	15.7	....	....	....	14	60	Art. resp	....	....	....	....
		60 A	47.6	7.15	7.0	55/62	14.5	....	....	....	6	48	Art. resp.	30.4	....	Least +	H

								Ammonia									
19 (13.2) ½ dose	.....	B	93	6.9	7.05	70 / 74	10.9	+	++	....	140	60	18	35.2	....	+	H
	15.1 c.c.; 1.36 gm.	preceded by 6 per cent. acacia, 16 c.c. per kilogram and 18 per cent. urea, 100 c.c. (total)										120	24				
		39 A	80	7.4	7.37	140 / 140	11.5	....	....	....	140	75	30 Irregular	34.8	....	+	H
		66 A	79	7.4	....	120 / 120	11.3	++	+ tr.	....	134	87	27	34.6	....	+	H
								Sodium Citrate, 2 per Cent.									
17 (6.1)	.....	B	82.9	7.2	....	54 / 58	11.9	+++	None	....	80	264	90	37.0	....	....	D
	12.3 c.c.; 0.25 gm.	preceded by 1 per cent. sodium chlorid, 200 c.c., and 1.1 per cent. sodium phosphate, 200 c.c.										120 (5 min. later) deep; slowed			Struggling		
		22 A	70.4	7.2	7.18	52 / 56	10.9	+	....	....	94	Irregular	Irregular; very rapid	....	Struggling	....	Lighter color
		68 A	.....	7.35	....	45 / 46	....	++	....	....	74	...	Irregular; very rapid	....	....	....	Lighter color
		91 A	.....	7.2	....	48 / 50	15.0	++	None	....	74	192	48 Irregular	36.4	....	....	Lighter color
								Sodium Iodid, 21.7 per Cent.									
13 (9.4)	.....	B	84.0	7.1	....	.. / 56	....	....	....	....	132	100	20	35.4	....	....	....
	5.3 c.c.; 1.15 gm.	preceded by 0.9 per cent. sodium chlorid, 50 c.c., and 50 per cent. dextrose, 50 c.c.									130	88	18	35.2	....	....	....
		5 A	66.9	7.0	....	..	....	....	....	....	130	81	18	35.2	....	....	....
		20 A	72.9	6.9	....	.. / 53	....	....	....	....							
		65 A	73.5	6.9	....	..	....	....	....	....	112	148	30	35.2	...	....	....
											90 (20 min. later)						

TABLE 6.—*Effects of Miscellaneous Agents.—(Continued)*

Dog No. and Weight, Kg.	Dose per Kilogram	Time of Blood Specimen, Min.	Hemoglobin, per Cent.	p_H Colorimetric	p_H Electrometric	CO_2 Content / CO_2 Capacity Per Cent. by Volume	Urea Nitrogen, Mg. in 100 C.c.	Lactic Acid, per Cent.	Sugar, per Cent.	Total Phosphate, Mg. in 100 C.c.	Blood Pressure, Mm. Mercury	Pulse Rate	Respiratory Rate	Rectal Temperature, C.	General Symptoms	Sedimentation Rate	Agglutination and Other Changes
22 (5.9)	.....	B	100	7.0	6.99	54/55	10.8	+	....	8.2	84	90	15	37.4	Collapse 43 min. before	+ marked	D; Ag
			90.5	7.05	....	46/..	10.9	+	....	7.8	...	96	16	...	Recovered 33 min.	+ less	Less D
	8.5 c.c.; 1.84 gm.	25 A	80	7.1	7.1	38/39	13.1	++	....	8.6	120 (10 min. after) 100	90	24	....	None	+	D
		48 A	85.5	6.9	6.37	34/38	12.4	++	....	6.0	40	...	...	37.0	Severe collapse	+	D
		52 A*	76.7	7.05	....	38/41	12.4	....	....	8.1	20	0	Stopped	....	Died	...	....
		37 A*	....	....	....	39/41	12.0	....	....	9.2	0	0	...	....	....	....	....
23 (8.2)	.....	B	100	7.2	....	48/48	10.5	0.025	....	....	84	180	36	37.2	....	None	....
	4.9 c.c.; 1.06 gm.	20 A	86.2	7.15	....	45/51	12.0	.....	....	....	114	180	36 Irregular	37.0	....	+	D; Ag
		43 A	90.1	7.1	....	48/49	....	0.05+	....	....	90	...	Irregular	....	....	+	D; Ag
Disodium Phosphate, 1.1 and 5 per Cent.																	
17 (6.1)	.....	B	88.9	7.0	7.19	34/46	12.2	++	Ammonia +	....	94	110	120	37.0	....	....	....
No morphin	32.8 c.c.; 0.36 gm.	(1.1 per cent.) preceded by 1 per cent. sodium chlorid, 200 c.c.									120 (7 min. later)						
		31 A	76.9	7.35	7.26	54/58	11.9	....	....	....	90	264	90 shallow	37.0	Tremors	None	D
		51 A	82.9	7.2	....	54/58	....	+	None	....	86	Irregular	...	36.2	Tremors stopped	None	D

20 (9.7)	.....	B	89.3	6.9	6.97	56/60	13.8	+++	++	....	80	166	24	36.6	....	+ marked	....
	5.12 c.c.; 0.26 gm.	preceded by 5 per cent. gelatin, 16 c.c. per kilogram									110 (4 min. later)						
		32 A	86.9	7.2	7.14	54/62	13.0	++	....	....	80	180	28	36.4	Twitching and continuous convulsions	None	....
	5.12 c.c.; 0.26 gm.																
		30 A	81.6	7.25	7.14	55/64	11.3	....	+	....	70	200	36	37.0	Twitching and continuous convulsions	None	....
		69 A	84.4	7.25	....	54/63	11.5	+	....	....	60	...	...	...	....	....	....
							Sodium Salicylate, 50 per Cent.										
12 (10.2)	.....	B	90.9	7.3	7.23	50/..	12.9	+	....	....	136	66	20	35.6	....	....	....
	0.98 c.c.; 0.79 gm.	preceded by 0.9 per cent sodium chlorid, 100 c.c.															
		7 A	88.9	7.2	7.23	..	13.5	....	....	....	110	80	24 deep	35.6	Twitching	+	D
		17 A	79.7	7.2	7.16	40/..	13.1	++	....	....	126	174	28 very deep	36.4	Convulsions	+	D
		32 A	69.1	7.0	6.93	..	13.7	++	....	....	120	...	...	....	Collapse	+	D
	Postmortem from heart			6.4	6.47	..	....	....			0 (2 min. later, sudden collapse; died)						
11 (6.0)	.....	B	100	7.2	....	54/55	....	+	....	....	120	96	24	37.0	....	....	....
	0.98 c.c.; 0.49 gm.																
		10 A	88.5	6.9	....	50/50	....	++	....	....	120	100	34	36.8	Twitching	+	D
		20 A	80.0	6.9	....	46/48	....	+++	....	....	130	100	34	....	Collapse	+	D

* Blood from right ventricle.
† Blood from femoral artery.

amin was responsible for the increase in lactic acid or whether it was an accompanying change of the gradual circulatory collapse that supervened is not known. The sugar tended to increase in both dogs and the phosphate remained practically unchanged. The sedimentation rate of bloods in both dogs increased and agglutination, hemolysis and darkening were present in both.

The following symptoms were observed: The blood pressure fell steadily to a shock level in Dog *27*, which received only arsphenamin, while it rose moderately during the course of an hour and then fell to the shock level in the peptonized dog. Figure 6 illustrates the response in Dog *27*. Pulse and respiration were variable. Artificial respiration had to be used as collapse supervened in Dog *27*, which received only arsphenamin. This animal also showed tremors. The rectal temperature fell in both animals.

Accordingly, higher therapeutic doses of arsphenamin produce definite and progressive blood and symptomatic changes in dogs.

Calcium Chlorid, 9 Per Cent. Solution.—Injections of this solution were made in two dogs, of whom one (Dog 18) had received 6 per cent. acacia solution and 10 per cent. sodium chlorid solution and the other (Dog 19), 6 per cent. acacia solution previously. The results are presented for what they are worth. Dog 18, starting out with a lower level of blood pressure (80 mm.) than Dog 19 (134 mm.) died of collapse at the end of twenty-four minutes after the injection, while Dog 19 lived without marked symptomatic changes, except for slowing of the pulse and respiration. Blood dilution occurred in Dog 18, and concentration in Dog 19. The carbon dioxid of the blood was reduced in both animals out of proportion to the changes in blood concentration (volume), the increased blood carbonate after the previous injection of bicarbonate in Dog 19 being especially reduced by the calcium chlorid injection. Odaira [53] reports reduction of carbon dioxid in blood with isotonic calcium chlorid solution as well as with hypertonic solutions, but not with other isotonic solutions. No effect on the acid-base equilibrium of the blood in dogs after intravenous injection of from 0.5 to 1.1 gm. of calcium chlorid is reported by Salvesen, Hastings and McIntosh; [54] but they state that the calcium caused an increase in phosphate. On the other hand, oral administration in dogs and man was found by these observers to produce uncompensated acidosis (p_H of 7.13 in a dog and of 7.14 in a patient with nephritis). Lactic acid was reduced in both of our dogs, and the p_H, urea and ammonia were practically unchanged. The sedimentation rate of the blood in both dogs, having been increased

53. Odaira, T.: Tohoku J. Exper. Med. **4**:253 (Feb. 3) 1924.

54. Salvesen, H. A.; Hastings, A. B., and McIntosh, J. F.: J. Biol. Chem. **60**:277, 1924.

by the preceding injections, was retarded after the calcium. That is, the suspension of the corpuscles was more stable after calcium. Hemolysis from the preceding agents remained unchanged, as might be expected. In some respects, therefore, calcium chlorid given intravenously was not detrimental, though it is true that one of the dogs suffered fatal collapse. Both dogs had been injected previously with acacia and other agents that cause blood and symptomatic changes, as indicated in the previous sections of this paper. Certainly in Dog 19 calcium chlorid did not contribute any important detrimental changes; on the contrary, it alleviated corpuscular injury, as indicated by the improved stability of the blood in both dogs. It is interesting to note that injection of calcium chlorid into guinea-pigs does not as a rule produce anaphylactoid reactions.

Calcium salts are used therapeutically to allay or prevent certain allergic reactions and to diminish vascular permeability. The following are some recent reports on intravenous dosage and effects in human subjects: Bruhl and Buc [55] report the use of from 20 to 100 c.c. of 3 per cent. calcium chlorid (3 gm. daily) in tuberculous patients with no resulting symptoms except nausea. Kawakami [56] reports the occurrence of headaches from the injection of from 5 to 30 c.c. of 1 per cent. calcium chlorid solution once or twice daily. Dschalalian [57] asserts that 0.1 gm. of calcium chlorid per kilogram is not dangerous. In F. von Müller's clinic at Munich, slow injections of 3 c.c. of 20 per cent. calcium lactate solution were reported to cause a small pulse and weakness. Von den Velden [58] advises only small doses of 5 c.c. of 1 per cent. calcium chlorid solution. Haffner [59] states that 20 c.c. of 3.4 per cent. calcium chlorid solution can be injected for twenty minutes with minor effects; that 3 c.c. of 3.4 per cent. solution (0.004 gm. per kilogram) causes a fall of blood pressure and respiratory stimulation in half a minute, and that 0.07 gm. per kilogram causes respiratory and cardiac standstill.

Copper Sulphate, 5 Per Cent. Solution.—Very small doses of this agent produce violent reactions in guinea-pigs and also in dogs. In Dog 30 the injection was uncomplicated by previous injections, but in Dog 32 there were previous injections of barium sulphate given intravenously and intracarotidly. However, Dog 32 was in good condition at the time of the copper sulphate injection and the results from both dogs agreed remarkedly well. The blood changes in both dogs were interesting and profound in certain directions considering the rapid onset of death (in seven and thirteen minutes). The blood concentration increased in Dog 30, and the increase from barium sulphate in Dog 32 became somewhat reduced, though not to normal; the hemoglobin remaining about 5.5 per cent. above normal. The p_H and carbon dioxid were considerably reduced. The urea, lactic acid and sugar also were

55. Bruhl and Buc: Compt. rend. Soc. de biol. **74**:880, 1913.
56. Kawakami: Tokyo med. Wchnschr., p. 2572, 1913.
57. Dschalalian: Dissertation, Munich, 1913.
58. Von den Velden, R.: Therap. Monatsh. **27**:685, 1913.
59. Haffner: Arch. internat. de pharmacol. **23**:37, 1913.

reduced, but not as much as the carbon dioxid. Phosphate was variable. These changes cannot be explained by the change in blood volume as indicated by the hemoglobin changes. The reduction in carbon dioxid and lactic acid cannot explain the decided tendency toward acidity (reduced p_H), and the phosphate was increased in one and reduced in the other dog. The bloods of both dogs showed an increased sedimentation rate and hemolysis, darkening in Dog 30 and agglutination in Dog 32. No coagulation of the bloods of both dogs occurred at the end of half an hour. They were not observed beyond that period.

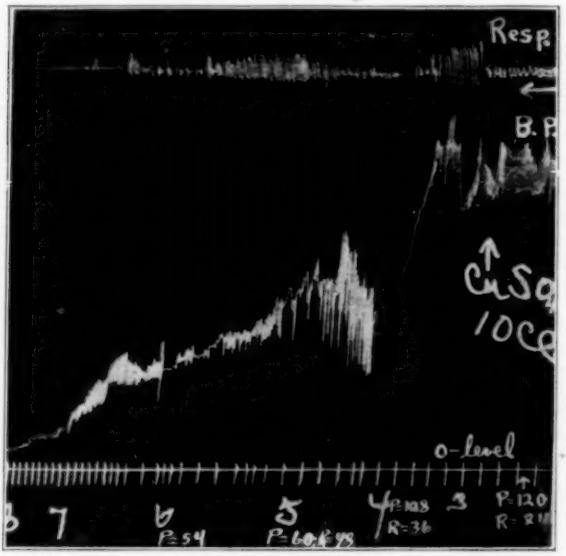

Fig. 7.—Effects of 5 per cent. solution of copper sulphate (0.049 gm. per kilogram) intravenously in Dog 30, which weighed 10.2 kg. *P*, pulse; *R*, respiration. Blood samples from femoral artery were taken at 6 and 7 and from the right ventricle at 8. Each stroke in the record equals twenty seconds.

The symptoms in the two dogs were similar. The blood pressure fell rapidly and progressively to the zero level, and the pulse and respiration were slowed. In Dog 30 the respiration stopped after the heart stopped, while in Dog 32 heart and respiration stopped together. Figure 7 illustrates the circulatory and respiratory changes in Dog 30. Convulsions were present in both animals, and in Dog 30 there was in addition considerable salivation.

It is concluded that copper sulphate produces marked blood and symptomatic changes in dogs.

Histamin, 0.01 Per Cent. Solution.—This was tried in an unmorphinized dog (Dog 26) which had previously received 20 c.c. of 0.1 per cent. agar solution. Several divided doses were injected. The blood changes consisted of: a slight though definite and progressive increase in concentration, fluctuation of the p_H between 7.2 and 7.15; an increase in carbon dioxid after the first five doses of the drug but thereafter a progressive and marked decrease to the end of the experiment, and definite decreases in urea, lactic acid and sugar. All the decreases occurred despite the slight tendency to increase in blood concentration (or reduced volume). On the other hand, the total phosphate progressively increased somewhat more than could be accounted for by the increased blood concentration. At times the sedimentation rate of the blood was increased, and hemolysis was present at the end of the experiment. There were no other symptoms besides a steady and marked fall of blood pressure from 106 to 46 mm. of mercury at the end of about an hour after the first five doses of histamin; then the pressure rose to 80 mm. at the end of about ninety-four minutes. The pulse was slowed and the respiration was irregular, being slowed and deepened and accelerated at different times. The concentrating effect of histamin on the hemoglobin of the blood has been reported by Underhill and Kapsinow.[60] The number of corpuscles also tends to increase. These changes might be expected on account of the relaxing effect of histamin on the capillaries with the increase in their permeability asserted by some investigators. Increased vascular permeability with escape of plasma might explain the diminutions in carbon dioxid lactic acid and sugar, but not the increase in phosphate and the fluctuating p_H. On the other hand, the decreases in carbon dioxid and lactic acid appeared to be greater than the degree of increased vascular permeability as indicated by the relatively small increases in hemoglobin or blood concentration. Nevertheless, the one experiment made by us indicates that under the conditions histamin definitely altered the blood chemically with respect to some important constituents. The circulatory changes were of the usual kind.

Peptone (Witte) 5 Per Cent. Solution.—Intravenous injection of this agent in dogs is known to produce shock somewhat analogous to that of histamin, but the results are variable. A high dosage usually is necessary. We used about one-tenth the dose frequently used for production of shock. Dog 27 received a previous injection of arsphenamin and its blood pressure was at shock level in the beginning. The injection in Dog 28 was uncomplicated by previous intravenous injections. Many of the blood changes in the two dogs were opposite; others were similar. Both dogs responded to a slight increase or practically no change in blood concentration at the end of half an hour after the

60. Underhill, F. P., and Kapsinow, R.: Am. J. Physiol. **63**:142 (Dec.) 1922.

injections, and both showed a tendency to dilution at the end of an hour. The previously arsphenaminized dog (Dog 27) showed considerable reduction in p_H and carbon dioxid, a small decrease in sugar, a temporary increase in urea and a progressive increase in total phosphate. The marked tendency toward acidity (reduced p_H) at the end of one half an hour might be explained by the unchanged and slightly decreased blood concentration. On the other hand, in Dog 28, the p_H was only slightly and temporarily lowered, the carbon dioxid was increased and the lactic acid, sugar and phosphate were decreased. The increase in carbon dioxid might explain the reduced p_H value at the end of one-half an hour, and yet the increase in carbon dioxid could hardly be accounted for by the slight increase in blood concentration (reduced blood volume). Although the blood changes in the two dogs are not consistent, it appears that considerable modification of the blood occurred. This is sustained by the increased sedimentation rate and agglutination which occurred in the bloods of both dogs. The hemolysis of arsphenamin in Dog 27 did not appear to be increased after the peptone injection.

The blood pressure in Dog 28, which received only peptone, remained unchanged but the pulse and respiration were slowed. In the previously arsphenaminized dog (Dog 27), the blood pressure fell about 23 per cent., the pulse was accelerated and respiration was slowed. No other symptoms were observed. It appears that peptone can produce considerable changes in the blood in the absence of marked symptomatic changes.

Sodium Bicarbonate, 9 Per Cent. Solution.—Injections of this solution were made in two dogs that had received intravenous injections of other solutions previously. As a result of the previous injections there was considerable circulatory depression in both dogs to start with. The bicarbonate injections resulted in temporary improvement of their conditions. The improvement was more marked and lasting in Dog 19, which had previously received 6 per cent. acacia solution and 18 per cent. urea solution and whose original blood pressure was 60 mm. of mercury, than in Dog 16, which had been previously injected with 40 per cent. hexamethylenamin and 18 per cent. urea solution and whose pressure was 14 mm. The principal changes consisted of: a moderate blood dilution; a marked increase in p_H, i. e., alkalinity; a marked increase in carbon dioxid and some increase in urea. Lactic acid was found to be definitely increased in Dog 19 (the only one tested for this). This was in agreement with the report of Macleod and Knapp,[19] who found an increase in lactic acid of the urine and blood after administration of bicarbonate to rabbits. Ammonia was reduced in the same dog. The increased sedimentation rate of the blood produced by other agents in Dog 16 was reduced by the bicarbonate. The hemolysis caused by the

previous agents was not altered as might be expected. The pulse and the respiration were accelerated in both dogs. The original low level of blood pressure in Dog 16 was considerably raised for half an hour, but at the end of one hour it fell to 6 mm.; while in Dog 19, the pressure remained practically unchanged during the same period.

Apparently, bicarbonate is a good corrective for tendencies toward blood acidity, since the p_H of the blood of Dog 16 was raised from 6.6 to 7.3 and then to 7.15, and of Dog 19 from 6.9 to 7.4 at the end of an hour. Simultaneously, the symptoms in these dogs injured by previous injections were improved; at least they were not made worse, which was the result of the intravenous injection of several other apparently nontoxic and inert agents in this paper. The beneficial results of sodium bicarbonate observed by us agree with previous reports of others on the benefits of this salt in collapse conditions, acidotic tendencies, etc. For purposes of this study, the results with bicarbonate furnish a contrast to those with other salts and crystalloids injected intravenously. As far as p_H and carbon dioxid are concerned, they may be regarded as furnishing a proof of the correctness of the analytic data of all other agents and, in the same sense as asphyxia, they were used on occasion to test the analytic methods employed.

Sodium Citrate, 2 Per Cent. Solution.—Citrate was injected into a dog that had received 1 per cent. sodium chlorid solution and 1.1 per cent. disodium phosphate solution. At the time of the citrate injection, the dog was somewhat depressed, as indicated by the level of 80 mm. of mercury blood pressure and hemoglobin of 82.9 per cent. The citrate caused some further dilution of the blood without important changes in p_H; a decrease in carbon dioxid that could be accounted for largely by the blood dilution; reduction in lactic acid as compared with the control blood; absence of ammonia, and variable urea. Immediately after the injection there was some struggling, but there were no tremors or convulsions. The blood pressure rose from 80 to 120 mm. and then fell to 94 mm. at the end of twenty-two minutes, and then to 74 mm. at the end of ninety-one minutes. The pulse rate was slowed, but respiration was very rapid and irregular soon after injection, remaining irregular though slowed at the end of ninety-one minutes. The citrate bloods appeared lighter in color than the control, which had been darkened from the previous injections. With the exception of some blood dilution and reduction in carbon dioxid, the changes produced by the citrate in this dog, that had received previous intravenous injections and was not in the best condition were relatively unimportant. Further injections were not tried.

Sodium Iodid, 21.7 Per Cent. Solution.—Solutions of sodium iodid of about this strength (from 20 to 21 per cent.) are advocated for intra-

venous use in human subjects; the dosage varies. The 21.7 per cent. solution is approximately equimolecular with 10 per cent. sodium chlorid solution and is distinctly hypertonic. In Dogs 22 and 23 the injections were uncomplicated with other intravenous injections, but Dog 23 received previous injections of 0.9 per cent. sodium chlorid solution and 50 per cent. dextrose solution. The blood and symptomatic changes were quite uniform in all the dogs.

Taking the blood changes first, there was considerable blood dilution, a tendency toward acidity (lowered p_H), and a reduction in carbon dioxid more or less proportional to the blood dilution. In Dog 23 there was some recovery from blood dilution at the end of forty-three minutes and with this an increase in carbon dioxid to the previous percentage by volume. Lactic acid and urea were definitely increased in the two dogs receiving iodid alone, while the phosphate of one of these (Dog 22) remained practically unchanged. Sugar was not estimated. Conway [61]

Fig. 8.—Effect of 21.7 per cent. solution of sodium iodid (1.84 gm. per kilogram) intravenously in Dog 22, which weighed 5.9 kg. *P*, pulse; *R*, respiration. Blood samples were taken from femoral artery at 5 and from heart at 7. Each stroke in the record equals twenty seconds.

has reported that sodium iodid as well as urea, sodium bicarbonate, disodium phosphate and sodium sulphate in isosmotic concentrations with blood and in the same quantities (30 c.c.) caused a marked fall of dextrose of the blood and total output in the urine of rabbits receiving dextrose injections. This was not true of isotonic sodium chlorid. In the two dogs observed by us, the increases in lactic acid apparently contributed to the lowering of the p_H. Increased sedimentation rate, agglutination and darkening of the arterial bloods were present in the two dogs receiving iodid alone. The records for Dog 13 were incomplete.

The blood pressure rose temporarily in Dogs 22 and 23, which received iodid alone, and then fell gradually, resulting in the collapse and death of Dog 22. In this dog the respiratory and pulse rates were accelerated during the increase in blood pressure, but in the other dog they remained unchanged, though the respiration was irregular. In Dog 13, which had previously received other solutions, the blood pressure

61. Conway, E. J.: J. Physiol. **58**:234 (Dec.) 1923.

fell gradually from 132 to 90 mm. at the end of sixty-five minutes, and the pulse and respiratory rates were slowed until blood pressure fell considerably (112 and 90 mm.); they were then accelerated markedly. As all the dogs were morphinized, the temperature fell. No other symptoms such as tremors or twitchings were recognizable. Nevertheless, one (Dog 22) receiving iodid alone died from the injection. There is no doubt, therefore, that 21.7 per cent. iodid solution in the doses used causes definite and considerable blood and symptomatic changes in dogs, and that these cannot be regarded as beneficial.

Disodium Phosphate, 1.1 and 5 Per Cent. Solution.—The 1.1 per cent. phosphate solution was injected into Dog 17, which had previously received 200 c.c. of 1 per cent. sodium chlorid solution intravenously, and the 5 per cent. phosphate solution was injected into Dog 20, which had been previously injected with 5 per cent. gelatin. At the time of the phosphate injections, both dogs had blood pressures below normal and their bloods were moderately diluted. The changes in the two were, however, in the same direction. As a result of the phosphate injection, the bloods were moderately diluted and the p_H increased from neutrality (6.9 to 7.0) to alkalinity (7.35 and 7.2). In Dog 17 the carbon dioxid was considerably increased, while it remained practically unchanged in Dog 20 (the carbon dioxid capacity being increased in Dog 20); urea fell slightly, and lactic acid and ammonia were reduced. Darkening of the blood occurred in Dog 17; the marked increase in sedimentation rate of blood in Dog 20 was prevented, and the increase was absent in Dog 17. With the exception of slight blood dilution, the changes in the blood appeared to be beneficial. As to the considerable increase in alkalinity, the effect was similar to that from bicarbonate; but the increase in carbon dioxid was much less.

The symptoms in Dog 17 consisted of tremors of fifty-one minutes' duration, in Dog 20 of twitching and continued convulsions lasting sixty-nine minutes. Immediately after the injections, the blood pressure rose considerably in both dogs; at the end of about half an hour it returned to the previous level, and then gradually fell during the tremors and convulsions. In Dog 17 the pressure fell only from 90 to 86 mm. at the end of fifty-one minutes, while in Dog 20 it fell from 80 to 60 mm. at the end of sixty-nine minutes. In these dogs the sustaining effect of convulsions on the blood pressure often found with strychnin and asphyxial convulsions was not evident. The pulse was accelerated in both dogs; in Dog 17 respiration was slowed and irregular, in Dog 20, moderately accelerated. The rectal temperature continued to fall in Dog 17; it rose in Dog 20, presumably (in part at least) because of the convulsions. Symptomatically, therefore, the disodium phosphate solutions produced definite effects. So far as they were determined, the

blood changes were in the direction of normality. No estimations were made of phosphate, sugar and calcium. The calcium ion might be important with phosphate and citrate.

According to Binger,[62] who has made such estimations in dogs, the calcium content of blood is decreased after alkaline and acid phosphate injections, and this investigator believes that the mechanism of toxicity is intimately bound up with the serum calcium. There are other views as to the mechanism of action, and the reports also vary as to the occurrence of symptoms of tetany. Binger did not observe tetany in his experiments. Greenwald[63] ascribes the toxicity of various alkaline solutions of sodium salts (containing the anions Cl^-, $HPO_4^=$, $H_2PO_4^-$, HCO_3^- and $CO_3^=$) to the sodium ions rather than to the alkalinity. According to Underhill, Gross and Cohen,[64] the acid and alkaline sodium and potassium phosphates injected intravenously into dogs and rabbits reduced the blood calcium and caused symptoms of tetany, while sodium bicarbonate produced tetany without lowering of calcium. Fujimaki[24] reports that intravenous injections from every twenty to forty minutes of 5 c.c. of 5 per cent. solutions of monosodium and disodium phosphates in rabbits lowered the carbon dioxid capacity of the plasma whether the solutions were used with or without epinephrin; the disodium phosphate did not affect the carbon dioxid during dextrose injection. Lactic acid formation from sugar is suggested as being important in these phenomena. However this may be, our results are not sufficiently complete nor free from complications to permit any conclusions with respect to the mechanism of the increased excitability. They may be used, however, in the same sense as the results with bicarbonate, i. e., as controls of the methods employed and as indicating the general correctness of the results obtained with other agents.

Sodium Salicylate, 50 Per Cent. Solution.—This solution is advocated for intravenous use in human subjects without adequate basis, and practically nothing is known of its effects on the blood. Collapse is known to occur from the use of salicylate intravenously. Large doses are the rule in the treatment of rheumatic fever. The minimal fatal intravenous dose for dogs is about 1 gm. per kilogram (Sollmann). We used 0.49 gm. per kilogram in two dogs, of whom one (Dog 12) had received previously 100 c.c. of 0.9 per cent. sodium chlorid solution intravenously. This would be a very high dosage for man, but we were not interested in determining the scope of intravenous dosage for man, because there is no good indication for its intravenous usage therapeutically, all the ordinary effects and therapeutic benefits being promptly obtained with oral administration of the drug. We sought to ascertain what, if any, changes in the blood, etc., would occur from adequate dosage and concentration, for if no changes occurred under these conditions, it might be assumed that no, or only unimportant, changes would occur from minimal doses. Striking results were obtained. Definite changes in the blood occurred long before circulatory collapse supervened, and both dogs died suddenly and unexpectedly at the end of thirty-two and twenty minutes.

62. Binger, C.: J. Pharm. & Exper. Therap. **10**:105 (Aug.) 1917.
63. Greenwald, I.: J. Biol. Chem. **54**:285 (Oct.) 1922.
64. Underhill, F. P.; Gross, E. G., and Cohen: J. Metabol. Res. **3**:679, 1923.

The symptoms consisted of twitching in both legs, in Dog 12 these were accompanied with a few mild convulsions or tremors, but these soon disappeared. In Dog 12 about seven minutes after the injection, the blood pressure fell about 20 per cent.; then it increased about 16 per cent. At the end of seventeen minutes, it was 126 mm. of mercury. It fell again slightly (being 120 mm.) at the end of thirty-two minutes, and two minutes later the heart stopped and the pressure fell to the zero level. The pulse rate had increased from 66 to 174 just before the heart stopped, and the respiration, which was considerably deepened, increased from 20 to 28. The temperature fell slightly just before the heart stopped. In Dog 11, the blood pressure rose from 120 to 130 mm. at the end of twenty minutes, when it suddenly fell to the zero level as the heart stopped. The pulse rate was accelerated from 96 to 100 and the respiration from 24 to 34 just before the heart stopped without warning. The temperature fell only 0.2 degree. The sudden and fatal collapse that unexpectedly supervened in both dogs was rather striking.

The bloods from both dogs showed darkening and increased sedimentation rate as compared with the controls. They resembled asphyxial bloods. Other changes in the bloods were considerable, and in Dog 12 there were progressive dilution, a marked tendency to acidity (lowered p_H), a reduction in carbon dioxid more or less proportional to the blood dilution, an increase in lactic acid, and an increase in urea. Phosphate and sugar were not estimated. All the blood changes were evident while the blood pressures were high and long before fatal collapse occurred. The blood of Dog 12 postmortem had the highest acidity of any blood in this study. There can be no doubt, therefore, that 50 per cent. salicylate solution in the dosage used definitely alters the blood and can cause symptoms which may result in unexpected circulatory collapse and death. Further killing of the animals for the study of smaller intravenous doses of salicylate was held unjustified.

COMMENT

The results with the different agents and solutions have been sufficiently discussed in the text and require no further discussion. They leave no doubt that a variety of agents physically and chemically different alter the blood and cause disturbances in physiologic functions. These occur with small doses and concentrations of certain agents, notably of agar. In other instances in our study, large doses and high concentrations were used though, as a rule, the concentrations correspond to those advocated for intravenous use in human subjects. With many such agents and solutions used intravenously for therapeutic purposes, the object and dosage are ill defined. Some practitioners administer large doses with the purpose of inducing marked systemic reactions.

Others use small doses. We have not studied the effects of small doses of the commoner agents, but this has been done recently by Handovsky.[65] Handovsky reports most interesting colloidoclastic effects after small doses of 0.35 gm. of sodium chlorid and of 1 gm. of dextrose injected intravenously in human subjects, and on addition of these agents to beef serum in vitro. The effects on both human and beef serum were practically the same, and consisted of increases in extractable cholesterol as a result of some influence (perhaps loosening up) of the salt and sugar on the colloidal structure of the serums. It appears that the cholesterol of the circulating blood suffered some change in state. It was also interesting that the extractable cholesterol appeared in subjects responding with physiologic disturbances as a result of the injections, and that the cholesterol increased the vascular tonus in excised organs. These results of Handovsky on human and beef serums are in line with our results on changes in the blood of dogs after intravenous injections. With the isotonic and hypertonic solutions of sodium chlorid, hypertonic sugar solutions, salicylate, iodid and hexamethylenamin, large doses appear to be the rule, presumably because the solutes are regarded as nontoxic, inert and harmless. Our results indicate that such assumptions are unfounded despite the inertness and lack of toxicity of the solutes in the ordinary sense and when given by mouth. In order to impress the uselessness and dangers of generalizations regarding disturbances or changes in vivo from inertness, etc., in vitro, we need only to point to the considerable disturbances and changes produced by agar, acacia and hexamethylenamin described in the text, let alone the profound effects of more active agents and the unsuspected effects of some others. The effects of agar in low dosage are so marked as to tempt one to speculate on the possible mechanism of the action involved.

Agar is an emulsoid colloid possessing carbohydrate properties and is relatively inert chemically, being unreactive and nontoxic when given by mouth, but physically it is known to be rather active. For instance, it agglutinates corpuscles in vitro and causes pulmonary emboli and thrombi in guinea-pigs. These effects would not be expected from its ordinary properties. However, in suspension and in solution, agar, like many other colloids, presents an extensive surface, and when it is introduced into the blood stream it acts as an extensive foreign surface. Presumably as a result of the exposure of the blood and endothelium and perhaps of other tissues to this extensive foreign surface, changes in the surfaces of various blood elements supervene. These are somewhat akin to those of the precoagulation and coagulation states leading up to agglutination, flocculation and such conditions in the blood.

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65. Handovsky, H.: Klin. Wchnschr. **3**:1354 (July 22) 1924; München. med. Wchnschr. **71**:708 (May 30) 1924; Deutsch. med. Wchnschr. **50**:1315 (Sept. 26) 1924.

The latter changes have been demonstrated in corpuscles and thrombocytes with many of the agents used in this study, and are themselves indicative of surface changes. As a result of, or accompanying, these changes, there are changes in osmotic pressure, ionic equilibrium, surface tension, etc., of cell surfaces and of the cells themselves. These changes in turn determine the changes in the functional activity of the cells. There probably are other changes, and their complexity probably is greater than is realized. Adequate evidence has been obtained by one of us (F. D.) that the surface effects on the corpuscles produced by a number of the agents here reported occur from mere direct contact with the blood, and that they occur in vitro quite as readily as in vivo.

Other evidence obtained in this laboratory supports the assertion of Lumière [52] that the reactivity of certain agents depends on the state of fineness of the particles, the reaction being more marked the finer the subdivision of particles, and mild or unobtainable with the same agent in coarse suspension. This evidence will be published in the near future. These results are cited in support of the notion of contact phenomena resulting from direct contact of agents with the blood on intravenous injection. Similar views have been expressed by other investigators, but it is believed that the results reported in this paper furnish a measure of proof that has been lacking for the assumed disturbance in physical and chemical mechanisms as a result of the contact.

Some have regarded the fundamental changes in terms of "colloidoclasis" or "hemoclasia," indicating some disruption of, or disturbance in, the colloids of the blood and tissues. Perhaps the terminology is unimportant as long as the fundamental nature of the mechanism is kept in mind for purposes of further study. Whatever the ultimate explanation of the anaphylactoid reactions and crises, the results of this paper indicate that blood and symptomatic changes occur from agents physically and chemically unrelated, some of which are relatively inert in the ordinary sense, and also that more profound changes in the blood can occur than current conceptions of regulatory mechanisms would indicate. When blood with its important normal respiratory functions for tissues in general is altered, its functions may be considerably altered, and hence also those of the tissues. This is only one reason among others why further studies along the line of this paper are needed, and it pertains especially to agents that are being injected intravenously into human subjects.

The general symptoms that supervene are no less marked than the blood changes. Variations, of course, occur. Unsuspected and sudden changes are frequently encountered. Reactions that supervene from intravenous injections occur during, or very shortly after, the injection, if at all. Therefore, our blood analyses were made over short periods after the injections. After adjustment sets in or defensive mechanisms

begin to operate efficiently, the crisis begins to pass or is unrecognizable. However, numerous possibilities exist owing to the numerous variables in the complex organism, all of which increase the difficulties of the problem. The changes in the circulation, respiration, etc., that occurred after many of the agents in this paper deserve further analysis from the physiologic standpoint. The immediate effects (for example, the sudden changes in blood pressure) may be accounted for by quite obvious and easily demonstrable gross disturbances, such as cardiac inhibition from vagus stimulation, reflex or direct, direct cardiac depression and vascular relaxation. But, of course, the ultimate changes that bring about the gross changes may still, and probably do, rest on a physical-chemical basis. Similar conceptions have been advanced not only for the action of such colloidal agents as agar but also for the actions of alkaloids, crystalloids, anesthetics and drugs in general. For this reason, studies of the character reported in this article are not only of practical interest but also of fundamental biologic importance. The bearing of the results here reported on the effects and limitations of nonspecific protein therapy and on the dangers of intravenous medication in general is obvious. These features, together with extensive summaries from the literature of objective changes of all kinds that have been reported or demonstrated in allergic reactions from various agents, have been previously dealt with by one of us [66] and need not be dealt with here. The burden of proof rests on those who assert that intravenous injections are not injurious or dangerous.

CONCLUSIONS

1. Intravenous injections in dogs of small and large doses of the following agents and solutions caused definite and important changes in arterial blood, accompanied as a rule by disturbances in physiologic functions: 10 per cent. sodium chlorid solution, 85 per cent. sucrose solution, 50 per cent. dextrose solution, 18 per cent. urea solution, 0.1 per cent. agar solution, 6 per cent. and 25 per cent. acacia solution, 5 per cent. gelatin solution, 3 per cent. barium sulphate suspension, 0.5 per cent. fullers' earth suspension, 5 per cent. copper sulphate solution, 9 per cent. calcium chlorid solution, 21.7 per cent. sodium iodid solution, 50 per cent. sodium salicylate solution, 0.33 per cent. arsphenamin solution and 5 per cent. peptone solution.

2. The principal blood changes were:

(*a*) A considerable though variable dilution immediately after injection, except after barium sulphate, peptone and histamin, which tended to cause concentration or to prevent dilution.

66. Hanzlik (Footnote 2); California and Western Medicine **23**:161 (Feb.) 1925.

(*b*) A tendency to acidity as indicated by considerable lowering of p_H values, reduction from 7.3 or 7.2 to 6.9 or 6.8 being common and the lowest value observed being 6.6. Changes from the original were influenced by ether anesthesia, collapse, etc. With some agents, the changes were temporary, with others permanent.

(*c*) Accompanying the tendency to acidity, there usually was a reduction in the carbon dioxid (total and alkali reserve) of the plasma, and the reduction appeared to be chiefly a function of the blood dilution. Since the concentration of total carbon dioxid was reduced, the reduction in p_H was not due to carbon dioxid and presumably not to blood dilution per se.

(*d*) Of the fixed acids, lactic acid was generally increased when the p_H was lowered, and it appeared to be concerned with the tendency to acidity in part at least. Total phosphate was inconstant or variable, but the analyses were incomplete.

(*e*) Ammonia was frequently increased with the increase in lactic acid. Sugar showed unimportant changes, and urea remained constant or showed unimportant fluctuations even after injections of 18 per cent. urea solution.

(*f*) Darkening of the blood together with an increased sedimentation rate, agglutination and hemolysis occurred after agar, acacia, urea, gelatin, barium sulphate, fullers' earth, arsphenamin, copper sulphate, sodium iodid and sodium salicylate, except that there was no hemolysis with iodid and salicylate and no agglutination with urea. Darkening and agglutination occurred with dextrose; darkening with phosphate and agglutination with histamin; hemolysis with 10 per cent. and 0.1 per cent. sodium chlorid solution, horse serum and histamin, and increased sedimentation with 10 per cent. sodium chlorid solution. Reduction or inhibition of increase in sedimentation rate was observed with calcium chlorid, sodium bicarbonate and sodium phosphate. These phenomena indicate surface changes in, and injury to, the corpuscles.

3. Disturbances in physiologic functions were indicated from changes in blood pressure, pulse and respiratory rates, ranging from moderate to profound and frequently resulting in collapse and sometimes in death. Tremors, twitching of muscles and sometimes convulsions were observed, together with occasional increases in temperature of unmorphinized dogs, salivation and diuresis. Deaths occurred from injections of 40 per cent. hexamethylenamin solution, hexamethylenamin and sucrose, acacia, agar, copper sulphate, arsphenamin, fullers' earth, calcium chlorid preceded by acacia and sodium chlorid, peptone, sodium iodid and sodium salicylate.

4. The blood and symptomatic changes were not due to the removal of small quantities of blood for analyses and were, therefore, due to

the injections themselves. Induced asphxia which was used as control of the method of estimating p_H invariably lowered the p_H values (tendency toward acidity).

5. Lowering of p_H values of the blood did not occur or occurred only irregularly after the injection of 0.9 per cent. sodium chlorid solution (p_H and carbon dioxid lowered in two out of five experiments) used as the vehicle or solvent for the various agents that were injected—0.1 per cent. sodium chlorid solution and Tyrode's solution. Increases in p_H (alkaline tendency) occurred after 1.5 and 5 per cent. disodium phosphate solution, 9 per cent. bicarbonate solution and 2 per cent. citrate solution, though some dilution and some or no reduction of carbon dioxid (except after bicarbonate, which increased it) of the blood occurred. Lactic acid tended to be augmented in alkaline bloods after the phosphate, bicarbonate and citrate, while ammonia tended to decrease. Other changes in the blood after these solutions have been given in Paragraph *2 f*.

6. All the changes in Paragraphs 2, 3 and 5 occurred in morphinized and unmorphinized dogs, with and without artificial respiration. Therefore, the blood changes appeared to be produced independently of the respiration, and resulted from direct contact of the solutions and agents with the blood and tissues.

7. It is suggested that the basis of the blood and symptomatic changes that were demonstrated, and which resulted from contact with a variety of agents physically and chemically unrelated, rests fundamentally on disturbances in important physical and chemical mechanisms of the blood and tissues.

8. The practical bearing of the results on nonspecific foreign protein therapy and on intravenous medication is indicated and has been discussed in previous articles.

THE LIPOID PARTITION IN BLOOD IN HEALTH AND IN DISEASE *

BERNARD L. OSER, M.S., AND WALTER G. KARR, PH.D.
PHILADELPHIA

The development in recent years of methods for the chemical analysis of the fatty constituents of blood has made possible the study of the partition of cholesterol and lecithin between the blood plasma and cells, a matter of great interest in view of the reported antagonism existing between these substances. This article is part of a group of studies on the concentration and distribution of these lipoids in normal and pathologic blood, and the relation of these factors to certain physicochemical properties of the red blood cells possibly dependent on them. It is also planned to study from this standpoint the balance between free and combined cholesterol. The investigation herein reported is concerned only with the lipoid partition in the blood of normal subjects and of patients suffering from a variety of conditions in which abnormalities in blood lipoids were expected.

It has been reported by Bloor,[1] Chauffard, Laroche and Grigaut,[2] and Gorham and Myers [3] that the total cholesterol content of normal blood plasma is only slightly above that of whole blood though generally somewhat greater than that of the corpuscles. The "lecithin" content of the plasma on the other hand is, according to Bloor, approximately half that of the corpuscles. The constancy that is understood to exist in the ratio of these lipoids in plasma and cells has been regarded as suggestive of a relationship between them, one exerting a neutralizing or balancing influence against the other. The lipoid content of the cells has been investigated by Bloor in a variety of pathologic conditions and has been found to be sufficiently constant to warrant the suggestion that to avoid confusion little attention should be directed to the figures for cell lipoids.[4]

In 1912 Grigaut and L'Huillier [5] published figures for the cholesterol distribution in forty-six pathologic cases in which the serum values varied between 71 and 840 mg. per hundred cubic centimeters, while the

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* From the Laboratory of Biochemistry of the Philadelphia General Hospital and the Graduate School of Medicine of the University of Pennsylvania.

1. Bloor, W. R.: J. Biol. Chem. **25**:577, 1915.
2. Chauffard, A.; Laroche, G., and Grigaut, A.: Compt. rend. Soc. de biol. **70**:336, 1911.
3. Gorham, F. D., and Myers, V. C.: Cholesterol Content of Blood, Arch. Int. Med. **20**:599 (Oct.) 1917.
4. Joslin, E. P.: The Treatment of Diabetes Mellitus, Ed. 2, Philadelphia, Lea & Febiger, 1917, p. 97.
5. Grigaut, A., and L'Huillier, A.: Compt. rend. Soc. de biol. **73**:202, 1912.

cell values remained within the limits of 110 to 195 mg. per hundred cubic centimeters. The latter was regarded as normal. This constancy of corpuscle cholesterol has since been confirmed by Bloor.

Later, Henes [6] reported normal figures for the distribution of cholesterol between serum and corpuscles, his conclusion being that 55.6 per cent. of the total cholesterol is found in the serum.

The likelihood of variations in the cell concentrations of lipoids, however, was indicated by the results of various investigators. Striking increases in the proportion of cholesterol ester in the cells were shown by Knudson [7] to take place during fat absorption. Pacini reported changes in the cholesterol distribution in anemia [8] and in gallstones.[9] Undernourishment induced by war diet was found by Rosenthal [10] to effect diminution in the cholesterol content of the cells. The work of MacAdam and Shiskin [11] on the relation of splenic function to anemia has shown variations to exist in the corpuscle cholesterol. It appears evident therefore that the question of lipoid distribution has by no means been definitely settled.

As Bloor points out, however, comparison of the work of various investigators is of little value unless due regard is given to the methods used, the time of drawing blood in relation to the last ingestion of food, and the lapse of time between collection of blood and analysis. A further factor of importance in this connection is the means employed for measuring the relative volume of the cells.

METHODS

The investigations of Brinkman [12] on the osmotic resistance of red blood cells would seem to indicate that washing the cells with isotonic salt solution removes some of the phosphatids. Consequently we should expect a difference between corpuscle "lecithin" values determined directly on washed cells and by calculation from the cell volume and values for whole blood and plasma. Partly for this reason and partly for its greater simplicity, we have chosen the latter procedure.

Blood was collected in oxalated tubes. Determinations were conducted as soon as possible after the blood was drawn, at least to the stage at which no alteration in lipoid composition or distribution could occur. Values for cell lipoids were obtained by calculation as indicated above.

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6. Henes, E.: Deutsch. Arch. f. klin. Med. **111**:122, 1913.
7. Knudson, A.: J. Biol. Chem. **32**:337 (Dec.) 1917; ibid. **45**:255 (Jan.) 1921.
8. Pacini, A. J. P.: Am. Med. **24**:92, 1918.
9. Pacini, A. J. P.: Med. Rec. **94**:441 (Sept. 14) 1918.
10. Rosenthal, F.: Deutsch. med. Wchnschr. **45**:571 (May 22) 1919.
11. MacAdam, W., and Shiskin, C.: Quart. J. Med. **16**:193 (April) 1923.
12. Brinkman, R.: Arch. néerl. physiol. **6**:451, 1922.

Cell Volume.—The blood, thoroughly mixed by inversion, was drawn into the graduated capillary tubes of the Daland hematocrit and centrifugated at 3,000 revolutions per minute for three minutes. The reading was made with the aid of a hand lens, and the tubes were then centrifugated another minute. Invariably there was no change in the reading. This method is quicker and, we believe, more accurate than the use of a graduated centrifuge for such measurements.

Cholesterol.—Cholesterol determinations were made by the method of Myers and Wardell.[13] In preliminary work with this method it was apparent that one-half hour extractions were not sufficient to remove completely all the cholesterol. In most cases one hour was found to suffice, so allowing a margin of safety all extractions were conducted for one and a half hours.

The development of the final color was allowed to proceed in the same light by which the readings were later to be made, as recommended by Bloor, Pelkan and Allen.[14] For colorimetric comparison a standard solution of cholesterol in chloroform was required in place of the artificial (naphthol green B) color standard that Myers and Wardell recommend for development of the color in the dark. It was necessary to prepare fresh standard cholesterol solutions from time to time, as the color developed from old solutions appeared more bluish than that from the chloroform extracts of desiccated blood.

Lipoid Phosphorus.—The determination of lipoid phosphorus was made on the alcohol-ether extract, 25 c.c. of which contained the lipoid fraction of 1 c.c. of whole blood or plasma. Five cubic centimeters of the extract were filtered into a pyrex test tube graduated at 5 c.c., and evaporated to dryness on the water bath. The residue was oxidized by digesting over a microburner with 0.5 c.c. of a mixture of equal parts of concentrated nitric and sulphuric acids, precautions being taken against overheating the tube. The oxidation took place in three distinct stages. First, the water and oxids of nitrogen passed off, then sulphuric anhydrid fumes; finally, these dissipated and the oxids of nitrogen reappeared. At this juncture the heating was discontinued and, after a few minutes cooling, a drop of 1 per cent. sucrose solution was introduced. The flame was again applied until the mixture just came to a boil. The sucrose solution is said to split up a refractory nitric acid-phosphoric acid complex which prevents the phosphoric acid from reacting colorimetrically. After cooling, from 1 to 2 c.c. of distilled water was added and the solution was again allowed to cool. To effect partial neutralization of the strong acid, 6 or 7 drops of concentrated sodium hydroxid

13. Myers, V. C., and Wardell, E. L.: J. Biol. Chem. **36**:147 (Oct.) 1918.
14. Bloor, W. R.; Pelkan, K. F., and Allen, D. M.: J. Biol. Chem. **52**:191 (May) 1922.

was carefully introduced, the tube was again cooled and distilled water added to the 5 c.c. mark. For the color development the Brigg's modification [15] of the Bell and Doisy [16] procedure for inorganic phosphorus was used.

COMMENT ON RESULTS

Normal Cases.—These included patients who were members of the hospital and laboratory staffs and some patients whose diagnosis indicated no disturbance in lipoid metabolism. The latter group included five cases of psychosis and two minor surgical cases. Among the hospital cases it was our experience that the average cell volumes fell about 5 per cent. below the limits usually regarded as normal, but only those

TABLE 1.—*Normal Cases*

		Whole Blood			Plasma			Cells		
Case	Cell Volume, per Cent.	Cholesterol, Mg. per 100 C.c.	Lipoid Phosphorus, Mg. per 100 C.c.	Ratio Chol. / Lip. P.	Cholesterol, Mg. per 100 C.c.	Lipoid Phosphorus, Mg. per 100 C.c.	Ratio Chol. / Lip. P.	Cholesterol Mg. per 100 C.c.	Lipoid Phosphorus, Mg. per 100 C.c.	Ratio Chol. / Lip. P.
1	39	183	15.2	12.04	200	11.9	16.80	156	20.3	7.68
2	37	175	14.2	12.32	203	11.3	17.96	127	17.8	7.14
3	43	160	11.7	13.67	189	9.2	20.54	121	15.1	8.01
4	42	142	12.1	11.73	155	8.8	17.61	124	16.7	7.42
5	40	140	13.3	10.52	146	9.7	15.05	131	18.8	6.97
6	45	148	11.3	13.10	169	7.4	22.84	122	16.0	7.63
7	48	146	11.7	12.48	193	8.8	21.92	96	14.8	6.48
8	43	154	15.1	10.20	169	11.3	14.96	135	20.2	6.68
9a	41.5	190	15.5	12.25	240	14.1	17.44	111	17.5	6.34
9b	42.5	165	14.0	11.79	238	11.7	20.54	66	17.1	3.86
10a	43	170	16.3	10.43	261	13.8	18.91	72	19.5	3.69
10b	45	140	13.7	10.22	199	10.4	19.13	68	17.8	3.82
10c	42	162	15.4	10.52	220	13.3	16.54	81	18.3	4.43
11	42	161	12.5	12.88	233	8.9	26.19	62	17.4	3.56
12	42	133	12.3	10.81	168	10.1	16.63	83	15.2	5.46
13	46	154	14.3	10.77	200	11.4	17.54	91	17.2	5.29
14	40	160	13.6	11.76	177	9.8	18.06	135	19.3	6.99
15	41	151	13.2	11.44	154	9.8	15.10	146	18.0	8.11
16	40	165	13.1	12.59	180	9.5	18.94	142	23.2	6.12
17	41	110	13.6	8.09	105	9.4	11.17	117	19.8	5.91
18	43	118	13.7	8.61	118	10.8	10.93	118	17.4	6.78
Averages.....		153.7	13.61	11.34	186.8	10.54	17.81	109.7	17.97	6.11

hospital cases were selected in which the cell volume was above 40 per cent. Perhaps the hematocrit method was responsible for the lower cell volumes in apparently normal cases.

From Table 1 it will be seen that both the cholesterol and lipoid phosphorus values for normal whole blood agree in general with those recorded in the literature. While the normal range for cholesterol in whole blood using the foregoing method is given as from 140 to 170 mg. per hundred cubic centimeters, occasionally values outside these limits were obtained. The figures for lipoid phosphorus in whole blood range somewhat higher than those usually reported. Bloor, for example, gives 12 mg. lipoid phosphorus (recalculated from "lecithin" on the basis of 4 per cent. phosphorus content) as the average for whole blood

15. Briggs, A. P.: J. Biol. Chem. **53**:13 (July) 1922.
16. Bell, R. D., and Doisy, E. A.: J. Biol. Chem. **44**:55 (Oct.) 1920.

of men, while here the average value is 13.6 mg. However, it is in the distribution of the lipoids that these results differ from those of others.

It is said that plasma cholesterol approximates though it is slightly higher than that of whole blood. Although the plasma cholesterol values presented are in practically all instances higher than the corresponding whole blood values, they can hardly be said to approximate them nor are the increments by any means constant. In the cases analyzed, an average of 68 per cent. of the total cholesterol of the whole blood was present in the plasma, as compared with from 55 to 60 per cent. reported by others. In other words, instead of the plasma cholesterol values being only slightly higher than corresponding whole blood values, these data show them to be anywhere from one to one and a half times as high. Consequently the concentration of cholesterol in the corpuscles in these cases is lower than usually reported and varies over a considerable range.

Cases 17 and 18 have been included in this series to illustrate the not infrequent occurrence of the blood of a healthy individual which is apparently normal in all respects except for its low plasma cholesterol content giving rise to low blood values.

Since the corpuscle values were calculated, as described above, they are subject to wider variation and greater possible error than those values obtained directly. Incidentally the corpuscle values reported by Bloor [1] for normal males were not calculated from actually determined cell volumes but were based on the average cell volume of Keith, Rowntree and Geraghty.[17] This average (43 per cent.) was obtained from only eighteen normal persons, the limits (from 32.7 to 49 per cent.) being obviously too extreme to warrant the use of the average in any single case.

The concentration of lipoid phosphorus in cells varies within narrower limits than in plasma. According to our data it is from one and a half to two times the plasma concentration, as compared to the 2:1 ratio obtained from Bloor's data. It is worthy of note that, except for a few instances, from 50 to 60 per cent. of the total lipoid phosphorus of whole blood is contained in the cells.

In the majority of the normal cases tabulated the ratio of cholesterol to lipoid phosphorus in the cells averaged about 6.1. Several cases showed ratios somewhat lower than this on account of diminished concentrations of cholesterol. The plasma ratios of cholesterol to lipoid phosphorus were less constant and averaged about 17.8. In terms of lecithin this means that there is somewhat more lecithin in the plasma than cholesterol. Bloor's data indicates the opposite, but it must be remembered that cholesterol values determined by his method are considerably higher than those determined by the method of Myers and

17. Keith, N. M.; Rowntree, L. G,, and Geraghty, J. T.: Arch. Int. Med. **16**:547 (Oct.) 1915.

Wardell. In normal whole blood the ratio of cholesterol to lipoid phosphorus was quite constant, averaging around 11.3. Those cases referred to above of normal blood with relatively low plasma cholesterol content showed the only marked discrepancy in this respect.

TABLE 2.—*Pathologic Cases*

		Whole Blood			Plasma			Cells		
Case	Cell Volume, per Cent.	Cholesterol, Mg. per 100 C.c.	Lipoid Phosphorus, Mg. per 100 C.c.	Ratio Chol. / Lip. P.	Cholesterol, Mg. per 100 C.c.	Lipoid Phosphorus, Mg. per 100 C.c.	Ratio Chol. / Lip. P.	Cholesterol Mg. per 100 C.c.	Lipoid Phosphorus, Mg. per 100 C.c.	Ratio Chol. / Lip. P.
Uremia										
51	20	280	20.2	13.86	346	19.4	17.83	15	23.5	0.66
52	20	185	13.7	13.50	184	11.0	16.73	190	24.5	7.76
53	18	300	18.1	16.58	354	19.5	18.15	56	11.7	4.79
54	22	200	12.9	15.50	196	11.9	16.47	214	16.4	13.05
55	9	225	15.7	14.33	237	11.9	19.91	100	54.4	1.84
56	35	147	14.1	10.43	171	11.3	15.13	103	19.4	5.32
56a	36	135	14.2	9.51	163	10.8	15.9	86	20.3	4.24
57	37	120	12.2	9.83	135	9.9	13.63	95	16.2	5.86
58	18.5	130	10.8	12.04	127	9.0	14.11	140	18.9	7.41
59	13	153	13.3	11.50	156	11.4	13.59	139	26.1	5.32
Diabetes										
60	38	172	15.4	11.16	212	11.9	17.82	108	21.1	5.12
61	49	150	19.0	7.90	215	15.9	13.52	82	22.2	3.69
62	36	134	13.1	10.23	160	9.8	16.33	80	26.7	3.33
63	43	183	15.2	11.97	274	13.2	20.76	60	17.9	3.35
64	33	150	14.9	10.06	171	12.0	14.25	106	20.9	5.07
65	42	140	13.5	10.37	133	10.2	13.04	150	18.1	8.29
66	40.5	161	16.4	9.82	175	11.4	15.35	141	23.7	5.95
67	34.5	142	11.7	12.13	120	8.1	14.81	182	18.5	9.83
68	23	651	.....	.....	809	.....	.....	0	.....	.....
Pregnancy										
69	32	207	16.7	12.39	230	16.1	14.28	139	18.1	8.78
70	35	222	19.0	11.68	245	15.7	15.60	180	25.2	7.14
71	30	225	17.9	12.56	300	22.5	13.33	50	7.0	7.14
72	33.5	242	16.6	14.58	283	22.6	12.52	161	4.8	33.54
73	30	175	15.9	11.01	200	13.3	15.04	117	20.3	5.76
74	33.5	230	17.8	12.92	285	16.9	16.86	119	19.7	6.04
Pernicious Anemia										
75	25	103	11.9	8.66	100	9.5	10.53	112	19.2	5.83
76	16	138	11.2	12.32	130	8.8	14.77	181	23.8	7.61
77	19	78	13.1	5.95	65	9.7	6.70	132	27.6	4.78
78	9	116	9.4	12.34	107	7.4	14.46	211	30.0	7.03
Banti's Disease										
79a	13	127	11.9	10.67	117	8.5	13.76	192	34.6	5.55
79b	16.5	151	14.5	10.41	131	11.7	11.20	254	28.4	8.94
80a	17.5	138	12.4	11.13	133	9.3	14.30	160	26.8	5.97
80b	23	120	12.6	9.52	95	7.8	12.18	204	28.7	7.11
81	15	150	13.3	11.28	156	9.4	16.60	113	35.2	3.22
Jaundice of Infectious Disease										
82	40	130	16.2	8.02	97	11.3	8.58	180	23.5	7.66
83	31	185	18.3	10.11	205	12.2	16.80	139	31.9	4.36
84	39	170	19.4	8.76	150	22.6	6.64	201	14.4	13.96
85	21	140	19.0	7.37	122	19.2	6.35	205	18.1	11.32
86	19.5	113	8.6	13.14	70	5.3	13.21	292	22.0	13.27
87	36	111	12.8	8.67	88	9.0	9.78	153	19.4	7.88
Obstructive Jaundice										
88	16	125	17.3	7.22	107	16.1	6.64	219	23.8	9.20
89	21	128	10.6	12.07	110	11.5	9.57	196	7.2	27.08
Cholelithiasis										
90	45	136	19.3	7.05	165	17.5	9.43	109	17.2	5.81
91	38	225	21.5	10.46	250	20.6	12.13	184	22.9	8.04

From these data it would appear difficult to assume a constant balance to exist between the amounts of cholesterol and lecithin in the blood and to accept the suggestion of an antagonism based on a quantitative relation between these substances.

Uremia.—The hypercholesterolemia encountered in some uremic cases may result from either an increase in both plasma and cells or low cell volumes accompanying normal or increased plasma values. In the plasma of these cases, the ratio of cholesterol to lipoid phosphorus is normal, but in those uremic cases with hypercholesterolemia a distinct disturbance in the absolute as well as the relative amounts of the lipoid substances is noted in the cells. Both cholesterol and phospholipoid vary widely and independently of each other.

Taking into account the varying degrees of anemia present, the fraction of total whole blood cholesterol and lipoid phosphorus contained in the plasma of these uremic cases is, on the average, above normal, in spite of the higher concentration sometimes encountered in the cells.

Bloor's [18] observations indicate an increase in fat and lipoid phosphorus in the corpuscles of nephritic patients, which he regards as due to the retarded assimilation accompanying the general metabolic disturbance associated with lowered alkali reserve. In agreement with the observations of others, we have found no relation to exist between the hypercholesterolemia of nephritis and the degree of nitrogen retention or carbon dioxid combining power. In their recent paper on fat metabolism in nephritis, Hiller, Linder, Lundsgaard and Van Slyke [19] suggest that the failure of the mechanism for transporting lipoids from the blood to the tissues may account for their accumulation in the blood. Whatever the causative factor of nephritic lipemia may be, however, it appears to effect not merely a piling up of fats but also a disturbance in their customary distribution.

Pernicious Anemia.—The hypocholesterolemia in these cases results from a diminution in concentration in the plasma with normal or even increased values in the cells, the latter having little influence on whole blood figures owing to the low cell volumes. There is evident in some cases a tendency toward increased concentrations of lipoid in the cells in pernicious anemia, although the proportion of the total whole blood lipoids found in the cells is below normal.

It may be remarked at this point that a tendency toward the accumulation of lipoids in the corpuscles is observed in most cases exhibiting anemia to a marked degree whether primary or secondary in origin. This would seem to indicate the existence of some relation between the lipoids of the cells and fat metabolism. It is suggested that the fat transport mechanism may be disturbed to the extent of placing too great a load on the relatively few blood cells. The corpuscles might be said to be overworked in their attempt to convert fatty acids to lecithin. Horiuchi,[20] however, failed to observe increased lecithin values in the

18. Bloor, W. R.: J. Biol. Chem. **31**:575 (Sept.) 1917.

19. Hiller, A.; Linder, G. C.; Lundsgaard, C., and Van Slyke, D. D.: J. Exper. Med. **39**:931 (June) 1924.

20. Horiuchi, Y.: J. Biol. Chem. **44**:363 (Nov.) 1920.

corpuscles of rabbits in experimental anemia and suggested that the resultant lipemia was due to the limitation of this function of the corpuscles.

These data do not agree with the statement of Bloor and MacPherson [21] to the effect that the blood lipoid values in anemia are normal so long as the percentage of corpuscles is more than one half the normal value.

It is well to recall, in discussing anemic blood, that the error in calculation of cell values increases in inverse ratio to the cell volume.

Banti's Disease.—The first two cases of Banti's disease studied manifest a divergence from the normal cholesterol partition in that there is a diminution in the plasma accompanied by a considerable increase in the cells, though because of the marked anemia whole blood values are low. MacAdam and Shiskin [11] report similar results on a case of Banti's disease. The lipoid phosphorus content of the cells in these cases is also abnormally high.

In each of these cases splenectomy was performed, following which the cholesterol lipoid phosphorus ratios dropped in the plasmas and rose in the cells, a decided absolute increase of cholesterol taking place in the cells of both cases. While the plasma cholesterol rose in only one of the cases, the plasma fraction of whole blood cholesterol fell in both cases. The second series of analyses were made one day after operation. The authors mentioned above have shown that the rise in plasma cholesterol following splenectomy is gradual and may even be preceded by a slight fall. We have noticed this diminution in phospholipoid as well as in cholesterol in Case 80. This, however, may have been related to an accompanying cirrhosis of the liver, which was the probable cause of death in this case. It may be recalled that Bloor mentions low vitality as a condition manifesting hypocholesterolemia.

The third case did not come under our observation until the seventeenth day after splenectomy was performed. The cholesterol partition is normal, though the lipoid phosphorus of the cells is quite increased.

Pregnancy.—Six cases are tabulated showing the rise in lipoids that occurs during the later months of pregnancy. The accumulation of lipoids is observed chiefly in the plasma, though in some cases, particularly in regard to cholesterol, the cells also participate. Considerable variation is found to exist in the phospholipoid concentration of the cells. A marked change in fat metabolism takes place, which alters the distribution as well as the concentration of lipoids in the blood. In this connection we are led to recall the observation of Meigs, Blatherwick and Carey [22] to the effect that the blood phosphatids are

21. Bloor, W. R., and MacPherson, D. J.: J. Biol. Chem. **31**:79 (July) 1917.
22. Meigs, E. B.; Blatherwick, N. R., and Carey, C. A.: J. Biol. Chem. **37**:1 (Jan.) 1919.

the precursors of milk fat, the prenatal increase thus being a preparation for the impending demand.

Jaundice of Infectious Disease.—The most characteristic feature of the lipoid picture in the blood in the type of jaundice found in infectious diseases is the distinct increase in the cell cholesterol. A diminution in the plasma cholesterol is generally observed also. The phospholipoids of both plasma and cells are usually normal, though occasional variations may occur. These changes in lipoid partition are masked when determinations are made on whole blood alone.

Obstructive Jaundice.—The cases of obstructive jaundice presented show a definite increase in cell cholesterol. It is a question, however, whether this may be related to the jaundice per se or to the marked anemia accompanying it. The lipoid phosphorus value in the cells of Case 89 is much reduced, an unusual occurrence in anemic blood.

Cholelithiasis.—The unreliability of cholesterol determinations as a means of diagnosis of gallstones is illustrated by the two cases reported, the diagnoses of which were confirmed at operation. Case 91 shows a hypercholesterolemia (whole blood) while Case 90 shows what may probably be regarded as a low normal. The partition of cholesterol is approximately normal in both cases. It is interesting to note that the lipoid phosphorus values in the plasmas are increased.

Diabetes.—While the whole blood concentrations of cholesterol and phospholipoid fall within normal limits, in most of our diabetic cases there is sometimes observed a distinct shift in the partition of cholesterol, usually in favor of the plasma. The cells may show increased phospholipoid values which may affect the whole blood figures, especially in a case of polycythemia like Case 61. Lipemia is not a common occurrence in the usual run of diabetic cases, especially those cases under treatment. Since the advent of insulin we have had difficulty in finding cases of diabetic lipemia. The one case given, that of a 6 year old girl with an extreme lipemia that persisted in spite of heavy doses of insulin, showed at one time 12.1 per cent. ether-soluble matter in the blood.

CONCLUSIONS

1. Variations in the lipoid content of normal human plasma and cells may occur over a somewhat wider range than previous work indicates.

2. Since no constant balance between cholesterol and "lecithin" appears to exist in normal blood, the theory of an antagonistic relation between these substances does not receive support.

3. Analyses of the blood in a series of pathologic cases show that considerable variation in the cell concentration of lipoid substances may occur, particularly in blood of low cell content.

CONGENITAL INTRACARDIAC FISTULAS

THEIR EFFECT ON THE DEVELOPMENT OF THE HEART *

EMILE HOLMAN, M.D.
CLEVELAND

The abnormal development of the heart that occurs in the presence of congenital anomalies has not been well understood and, consequently, not adequately explained. The following excerpt from a recent standard textbook [1] illustrates the difficulties of explanation presented by the patent septum:

> In an uncomplicated patent interventricular septum the blood seeks its way through the patency, *flowing back and forth between the two ventricles*, and if the opening is small the pressure between the right and left ventricles may remain equal, giving rise to no symptoms. In the majority of cases, however, the pressure in the two chambers is unequal. Thus *part of the blood is left over during the diastole, first in one side, then in the other. This in turn causes dilatation and finally hypertrophy.* Usually, enlargment of the right ventricle precedes that of the left, and complications may result from the accumulation of the blood in the chambers.

In our studies on peripheral arteriovenous fistula [2] and on the patent ductus arteriosus,[3] a very intimate interrelationship between increased minute volume flow and cardiac enlargement was demonstrated. Additional corroborative information as to this interrelationship was obtained from a study of such developmental anomalies of the heart as accompany patent interventricular septum, patent foramen ovale, cor triloculare, and the associated abnormalities of pulmonary and aortic stenosis. In the presence of these intracardiac fistulas, the volume flow of blood through the heart is unequally distributed among the several chambers, and theoretically the developmental response in these chambers should be commensurate with the volume flow through them. Clinical evidence in favor of this view is provided by the following cases of congenital anomalies of the heart:

REPORT OF CASES

CASE 1 (Fig. 1).[4]—A girl, aged 8 months, died from marasmus after being in the hospital for two days. The past history was one of malnutrition.

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* From the Department of Surgery, Western Reserve University School of Medicine.

* Presented before the Clinical Society of Surgery, Oct. 18, 1924.

1. Seham, Max: Congenital Heart Malformations, in Abt, I. A.: Pediatrics, Philadelphia, W. B. Saunders Company, 1924.
2. Holman, Emile: Experimental Studies in Arteriovenous Fistulas, Arch. Surg. **9**:872-879 (Nov.) 1924; Heart **9**:337-341 (Dec.) 1924.
3. Holman, Emile: Certain Types of Congenital Heart Disease Interpreted as Intracardiac Arteriovenous and Veno-Arterial Fistulae, I, Patent Ducus Arteriosus, Bull. Johns Hopkins Hosp. **36**:61-80 (Jan.) 1925.
4. Cautley: Two Specimens of Congenital Heart Disease, Proc. Roy. Soc Med., Section Dis. of Child. **2**:34-36, 1908.

anorexia, constipation and bottle feeding. The child was small and wasted, with an earthy complexion, a prominent sternum, beaded ribs, and hypertrophied heart without cardiac murmur, and an enlarged liver and spleen. There were no attacks of lividity or cyanosis.

At necropsy the heart was large and globular, weighing 4 ounces (113.4 gm.). Superficially, it showed no indication of two ventricles, and the wall felt the same thickness throughout. The auricles were normal. The foramen ovale was closed. The auriculoventricular valves appeared normal and competent. The ventricular cavity was large and globular and devoid of a septum, but exhibited a thick convex arch of muscle which separated the aorta and the pulmonary artery and roughly divided off a small left ventricle, the infundibular portion, from the large, dilated right ventricle into which both auricles opened. The pulmonary artery was much dilated above its origin as far as the origin of the two main trunks. The ductus arteriosus was of small size, and could not be regarded as pervious. The dilatation of the pul-

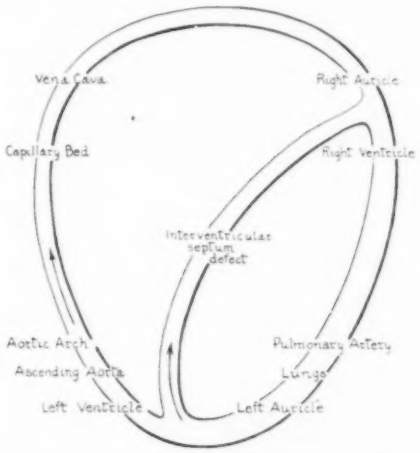

Fig. 1.—Patent septum acting as an arteriovenous fistula; no cyanosis; marked hypertrophy of right heart and dilatation of pulmonary artery; left ventricle and aorta small.

monary artery was no doubt due to the large amount of blood driven into it at each systole. The aorta was small and received little blood. This accounted for the poor development of the child.

The patent septum in this case served as an arteriovenous fistula transmitting oxygenated blood from the left side of the heart to the right. There was undoubtedly a mingling of venous and arterial blood in the right ventricle, but the blood received by the left ventricle through a "normal auriculoventricular valve," though small in amount, was fully oxygenated; consequently, the systemic circulation received only arterial blood. This is confirmed by the clinical description of an earthy complexion and by the absence of cyanosis. The cor triloculare, without cyanosis, has been cited as evidence against the admixture theory of

cyanosis, but it is evident that such an interpretation is not valid. The difference in development between the small left ventricle, with its correspondingly small aorta, and the large right ventricle, with the dilated pulmonary artery, is attributed to the difference in volume flow of blood through them.

Case 2 (Fig. 2).[5]—A boy, aged 15 months, had fairly well marked cyanosis. There was no clubbing of the fingers and toes. He suffered severe attacks of pain in the chest. The apex beat was forcible in the fifth space. There was cardiac dulness 1 inch (2.5 cm.) to the right of the sternum and upward to the third costal cartilage. A rough systolic murmur was heard all over the precordia, loudest at the third costal cartilage and the third space close to the left border of the sternum, conducted upward toward the left shoulder.

At necropsy the right auricle was greatly distended, and the left very small. No patent foramen ovale was present. The right ventricle was dilated

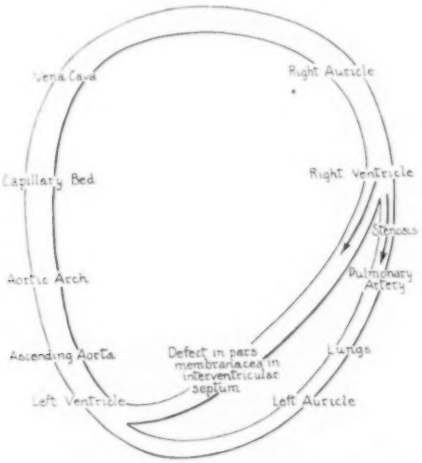

Fig. 2.—Septum defect acting as a veno-arterial fistula; cyanosis prominent; right auricle dilated; left auricle small; right ventricle dilated and hypertrophied; left ventricle also hypertrophied.

and its walls were greatly hypertrophied. There was stenosis of the pulmonary artery and of its orifice. The left ventricle was dilated and its walls were hypertrophied. There was an opening in the interventricular septum just below the aortic valves in the pars membranacea.

Case 3 (Fig. 2).[6]—W. P., aged 20, had always been blue, but was considered to be fairly strong till puberty. At the age of 17, he had two slight attacks of rheumatism; he became of a deeper blue color and suffered from dyspnea. He was very cyanosed and had very bulbous fingers. A systolic murmur was audible all over the cardiac area, somewhat variable from time to

5. Sawyer, J. E. H.: Congenital Malformations of the Heart, Pulmonary Stenosis and Patent Interventricular Septum, Birmingham M. Rev. **66**:153-168, 1909.

6. Rolleston, H. D.: Congenital Heart Disease; Pulmonary Stenosis; Patent Septum Ventriculorum, Tr. Path. Soc. London **43**:32-33-34, 1892.

time, but loudest over the fourth interspace to the left of the sternum. No thrill was ever felt. Death occurred from hemoptysis and phthisis.

At necropsy the heart weighed 20 ounces (567 gm.). The right auricle was both dilated and hypertrophied. The tricuspid valve was thickened. The right ventricle was hypertrophied so as to resemble the left ventricle, and was somewhat dilated. The pulmonary valves were welded together to form a funnel projecting up into the small thin walled pulmonary artery. The left auricle was normal and the fossa ovalis intact. The mitral valve was thickened and the left ventricle hypertrophied, but of about equal thickness with the right. The interventricular septum in the situation of the "undefended space" was patent. The circumference of this communication between the ventricles was 2⅞ inches (7.1 cm.), and its edges were rounded. The aortic orifice was directly over the septum, and that vessel may thus be said to have arisen from both ventricles. The aortic valves were somewhat thickened. The ductus arteriosus was obliterated and small.

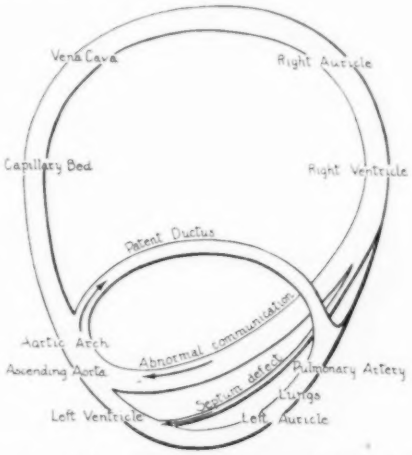

Fig. 3.—Abnormal opening between right ventricle and aorta acting as a veno-arterial fistula; patent ductus an arteriovenous fistula; cyanosis marked; right auricle dilated and hypertrophied; right ventricular wall thicker than left; pulmonary artery small; aorta very large.

In Cases 2 and 3, the patent septum may be designated as a veno-arterial fistula transmitting unoxygenated blood from the right to the left sides of the heart, and in each instance cyanosis was a prominent feature. In each the right auricle was dilated and hypertrophied as compared to the left, which was "very small" or "normal." This difference is interpreted as the response to the stimulus of an increased flow through the right auricle as compared to the left. It was noted in each case that the two ventricles were hypertrophied and dilated, the right being equal to the left in size and development. The aortic orifice was noted in Case 3 as arising from both ventricles. Undoubtedly, the

right heart was responsible, equally with the left, in supplying the systemic circulation with blood and in overcoming the systemic peripheral resistance.

Case 4 (Fig. 3).[7]—A girl, aged 11½ months, was first noted to have cyanosis at 6 months; this had been continuous more or less thereafter, with paroxysmal exacerbations. There was violet coloration of the hands and feet. No thrill was present. The pulse was 144. The respirations were tranquil. There were a systolic murmur and edema of the feet. Hemoglobin totaled 120; red blood cells, 6,417,000. There was dyspnea on the slightest exertion. The liver was enlarged. When the child cried there was intense cyanosis; this became more frequent and more intense in the last days of life. Death occurred in an attack of dyspnea and cyanosis.

At necropsy the heart was greatly enlarged, particularly on the right, which formed the apex. The heart musculature on the right was more firm than on the left. The musculature of the right auricle was hypertrophied, its cavity

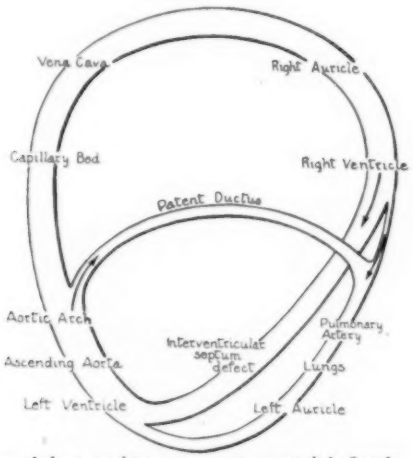

Fig. 4.—Septum defect acting as a veno-arterial fistula, patent ductus as an arteriovenous fistula; cyanosis prominent.

dilated. The thickness of the right ventricle was from 7 to 8 mm.; the thickness of the left ventricle, 6 mm. In the arch was the large opening of the ductus in communication with the pulmonary artery. The opening between the pulmonary artery and the right ventricle was completely obliterated. There was a free opening from the right ventricle into the aorta; this opening was much larger than that with the left ventricle. The opening between the ventricles was as large as a small pea. The aorta was very large—4 cm. in circumference. The ductus was 6 by 3 mm. The pulmonary artery was much smaller than the aorta, and the wall was thinner.

Case 5 (Fig. 4).[8]—A boy, aged 9, was apparently in perfect health until 7 years old. About two years before this examination, he experienced dyspnea,

7. D'Espine and Mallet, H.: Du Malformation Congenitale du Cœur avec Cyanose Paroxystique, Rev. de méd. **28**:941-949, 1908.

8. Johnston, James: Cyanosis with Deficiency of the Interventricular Septum, Brit. M. J. **2**:351, 1872.

pain across the chest and dizziness for the first time. He then had his first series of epileptic fits, and from that time on gradually became more cyanotic. The face and extremities were very blue. The lips were purple. The conjunctival membrane was congested. The apex beat was normal in position with a heaving rather than an accentuated impulse. There was a loud systolic murmur, harsh and rough in character, most distinct in the midsternum. This bruit was barely perceptible at the apex of the heart but was limited to a space on the sternum opposite the fourth left rib cartilage. There was no regurgitation in the veins. The bruit was most distinct in the horizontal position and frequently disappeared when the patient raised upright; but when this occurred he experienced a faintness and precordial pain, and then was convulsed. The lungs were fully distended. There was no increase in the number of respiratory movements, no cough, no expectoration, and he did not spit blood. The liver was not enlarged. The kidneys acted well. The first fit was noticed in the hospital about eight days after admission and fits then occurred at uncertain intervals until a week before his death. They were frequent during the last

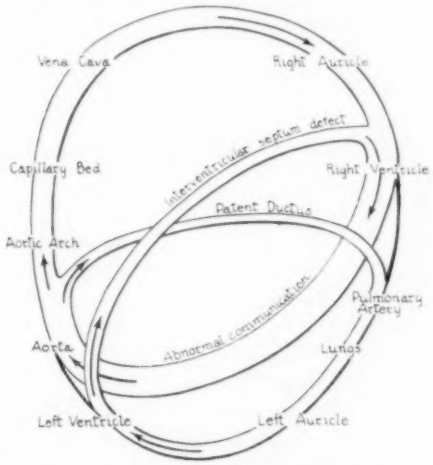

Fig. 5.—Transposition of aorta to arise from right ventricle; cyanosis marked from birth; right auricle greatly dilated; left auricle undeveloped; right ventricle much hypertrophied; left ventricle small; aorta large.

week of life, with painful intermissions and deep cyanosis. About ten days before death symptoms of pulmonary congestion came on with cough and frothy expectoration, but no blood. Death occurred after a series of fits.

At necropsy every organ was found deeply congested with dark fluid blood. In the right auricle the inferior and superior cavae were normal in size and position. The foramen ovale was closed. The tricuspid orifice was perfect and the valves were healthy. In the right ventricle the pulmonary arterial orifice was so small that it hardly admitted an ordinary probe. Immediately above and to the right side of this orifice, the aorta could be felt opening into the ventricle. The septum ventriculorum was deficient; the finger could be pushed with ease into the aorta from either the right or the left ventricle. The aorta seemed to be placed over both ventricles so as to receive the blood from both as from a common cavity. The ductus arteriosus was patent in its normal position and the diameter of an ordinary goose quill, in length about one-eighth inch (0.3 cm.). There can be no doubt that this state of the heart was congenital.

Cases 4 and 5 presented both a veno-arterial and an arteriovenous fistula in the same heart, the patent ductus in each case acting as the only pathway for the flow of blood through the lungs, and transmitting a mixture of arterial and venous blood from the aorta to the pulmonary artery. An abnormal communication transmitting such a mixture might properly be designated as a "veno-arteriovenous" fistula. In Case 4 the abnormal opening between the right ventricle and the aorta, and in Case 5 the septum defect acted as veno-arterial fistulas transmitting purely venous blood from the right heart into the systemic circulation, the volume of unaerated blood entering the aorta being greater than the volume of oxygenated blood from the left heart. This admixture of arterial and venous blood, with a preponderance of the latter, explains the high degree of cyanosis in each case. There is definite evidence, as will be discussed in a later article, that there occasionally occurs in the course of years a gradual narrowing and, finally, obliteration of the abnormal patent ductus widely open at birth, and it is to this fact that we attribute the ill health and cyanosis that followed seven years of normal good health in Case 5. As long as the patent ductus remained relatively large and transmitted a large amount of blood to the lungs for aeration, there was no cyanosis, and the oxygen interchange was sufficient for normal needs.

Case 4 also illustrates well the response of the heart chambers to the increased flow of blood through them, as evidenced by the dilatation and hypertrophy of the right auricle, and the greater thickness of the right ventricle as compared to the left. The right ventricle, in addition to its normal function, had, indeed, usurped part of the work of the left heart of propelling the stream of blood into and through the systemic circulation, and in consequence was proportionately more developed than normal.

Case 6 (Fig. 5).[9]—A boy, aged 4, had been livid from birth and subject to frequent pulmonary attacks. He was small for his age and presented the signs of extreme cyanosis, with great clubbing of the ends of the fingers and toes. There was some bulging at the lower part of the sternum and to the right of the normal cardiac area, with a forcible diffused cardiac impulse, chiefly on the right side of the sternum. The apex beat was most plainly visible about three-fourths inch (20 mm.) below the right nipple. No bruit could be heard at any time, but the first cardiac sound was doubled and extremely forcible, and the second sound everywhere accentuated. Air entered feebly at the extreme bases of the lungs. Death followed convulsions.

At necropsy the heart was larger than natural, the transverse diameter being almost as large as the vertical. The right auricle was enormously distended

9. Habershon, S. H.: Congenital Malformation of Heart and Kidneys; Obliteration of Pulmonary Artery; Aorta Arising from the Right Ventricle; Imperfection of Septum Ventriculorum; Lungs Supplied from Aorta by a Ductus Arteriosus Dividing Into Right and Left Pulmonary Branches; Horse-Shoe Kidney, Tr. Path. Soc. London **39**:71-74, 1887-1888.

and filled with black fluid blood. In situ only one vessel (the aorta) could be seen arising from a central position. When the cavities of the heart were opened, the right auricle was found to be dilated and thin walled; the vessels entered it normally, and the foramen ovale was closed. The right auriculo-ventricular valves were healthy and competent, but imperfectly divided into two unequal sized curtains. The right ventricle was enlarged and its walls were much hypertrophied. The aorta arose directly from the right ventricle, and was of unusually large size. The sigmoid valves were very large and loose. Immediately below their attachment was an imperfection in the base of the septum of a size sufficient to admit the little finger. The aorta must, therefore, have received blood directly from both ventricles during life. The left ventricle was of small size, it walls hypertrophied, but not to the same extent as the right. There was a normal appearing opening into it from the left auricle through a bicuspid valve, and a communication through the septum with the cavity of the right ventricle and with the aorta. No vessel opened from the left ventricle, and there was no trace of a pulmonary artery from

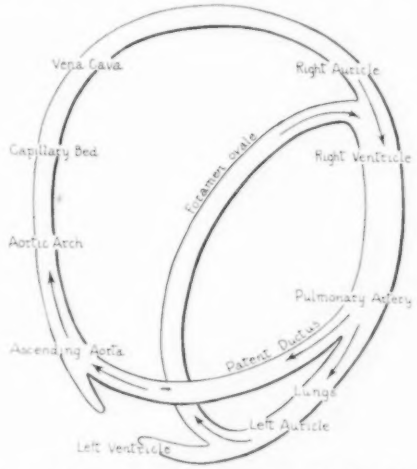

Fig. 6.—Right ventricle dilated and hypertrophied; left ventricle greatly reduced; pulmonary artery 29 mm. in diameter at its origin, 14 mm. in diameter after giving off patent ductus.

within the ventricle. The left auricle was atrophied and reduced to a venous sinus, into which the pulmonary veins opened. The left appendix auricle was present, but was very small. The aorta was of unusually large size. Immediately beyond the point where the great vessels were given off it became suddenly constricted to about half its previous dimension. The lungs were supplied from the aorta by means of a short trunk about one-fourth inch (0.6 cm.) in diameter, which was evidently the ductus arteriosus, and divided into a right and left pulmonary artery. Joining the right pulmonary branch was a vessel which could be traced to the heart to a point on the left of and a little anterior to the aorta. It was there lost in the muscular wall of the right ventricle. The lungs were congested but there was no collapse.

The lividity from birth in this patient is readily explained by the direct transfer of venous blood from the right heart into the systemic circulation. Only a minor portion of the circulating blood passed

through the patent ductus for aeration in the lungs. The differences in size between the enormously dilated right auricle and the atrophied left auricle, between the small left ventricle and the large hypertrophied right ventricle, are dependent on the unequal volumes of blood flowing through them.

CASE 7 (Fig. 6).[10]—A boy, aged 3 weeks, had dyspnea. Death occurred by suffocation.

At necropsy the right half of the heart appeared to be greatly hypertrophied. The right ventricle was greatly dilated. The pulmonary artery above the valves measured 29 mm. across; after the origin of the ductus botalli, 14 mm. The foramen ovale was open and broad. The circumference of the mitral opening measured about 22 mm. Below the mitral was found the greatly reduced left ventricle. The pulmonary artery communicated with the aorta by a 15 mm.

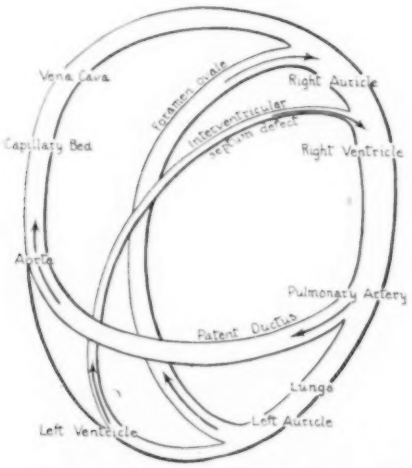

Fig. 7.—Foramen ovale and patent septum acting as arteriovenous fistulas, the patent ductus as a veno-arterial fistula; right auricle larger than left; right ventricular wall three times thicker than left. The patient had had cyanosis from birth.

long ductus botalli. The aorta measured, after the opening of the ductus botalli, 15 mm.; on the cardiac side of this opening, 10.5 mm., and before giving off the vessels of the head and neck, 7.5 mm. No communication between the aorta and the heart cavities was found. The heart weighed 41 gm.

Case 7 illustrates in a striking manner the interrelationship between the size of the large vessels and the volume flow of blood through them. The diameter of the pulmonary artery was exactly halved after giving off the patent ductus, and the aorta was considerably enlarged beyond the entrance of the ductus.

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10. Martens, Gustav: Zwei Fälle von Aorten Atresie, Virchows Arch. f. path. anat. **121**:322-335, 1890.

Case 8 (Fig. 7).[11]—An infant, 6 months old, was small and underdeveloped. The pulse was 130. The child was dyspneic and very cyanotic from birth. The heart sounds were clear; no thrill was heard.

At necropsy the heart was one and one-half times as large as the fist. The left auricle was smaller than the right. There was no aorta from the left ventricle. The right ventricle formed the apex of the heart. The musculature of the right was thicker than the left. The pulmonary artery was 1.4 cm. broad. The usual large arteries came from this pulmonary artery. The ductus was 6 mm. in diameter. There were two branches to the lungs. The foramen ovale measured 5 by 3 mm. The left ventricular wall was from 3 to 5 mm. thick. The right ventricular wall was from 0.8 to 1.2 cm. thick. There was a "pfennig stück groszes" round opening between the two ventricles. Cyanotic induration of organs was present.

Case 9 (Fig. 8).[12]—A boy, aged 5 months, was a patient from the age of 3 weeks. The child was cyanosed, sometimes deeply, sometimes only slightly. It had no clubbing of the fingers or toes. It was always very short of breath, its respirations being usually about 60 per minute. Its pulse varied from 160

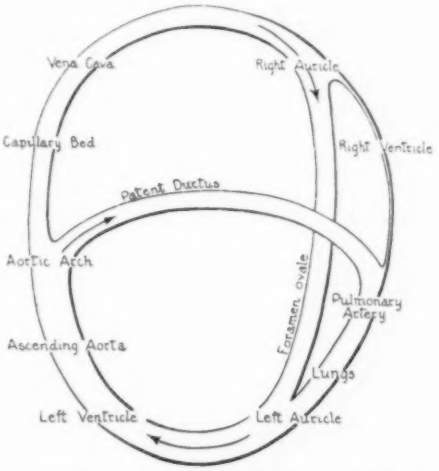

Fig. 8.—Foramen ovale acting as a veno-arterial fistula, the patent ductus as an arteriovenous fistula; cyanosis present; right auricle dilated and hypertrophied; right ventricle undeveloped; left ventricle abnormally dilated and hypertrophied.

to 180. It never sucked very well. The first and second heart sounds were audible. There was no murmur at any time. The general character of the sounds approached that of the sounds of the fetal heart. The ultimate cause of death was pulmonary catarrh.

At necropsy the aortic arch was somewhat disproportionately large, and arose from an abnormally large and thick left ventricle. A mitral valve with a partial third flap opened into the left ventricle from a left auricle of about normal size. The mitral flap bore the normal relation to the aortic valves,

11. Bókay, Z.: Ein Fall von Persistierendem Truncus Arteriosus Communis bei einem 6 Monate Alten Säugling, Jahr. f. Kinderh., Berlin **80**:327-334, 1914.

12. Moore, Norman: Congenital Malformation of Heart, Tr. Path. Soc. London **43**:31-32, 1892.

which were also normal. The foramen ovale was open, and the right auricle was of double the normal capacity and thickness. There was no true tricuspid valve, but only an opening the size of a common pin, which led into a right ventricle not larger than two pin heads. This had no communication with the left ventricle. A minute pulmonary artery without valves issued from the right ventricle. This pulmonary artery grew much wider as it approached the ductus arteriosus, which was patent and nearly as wide as the pulmonary artery.

The circulation was chiefly, if not entirely, carried on by the single ventricle, the pulmonary blood stream being directed through the ductus arteriosus from the aorta. The blood from the hypertrophied right auricle was driven into the left auricle, and thence, mixed with the aerated blood from the lungs, went through the wide mitral orifice into the left ventricle. There was no unmixed blood current. The arteries from the arch of the aorta were normal. The other viscera of the chest and abdomen showed no abnormality.

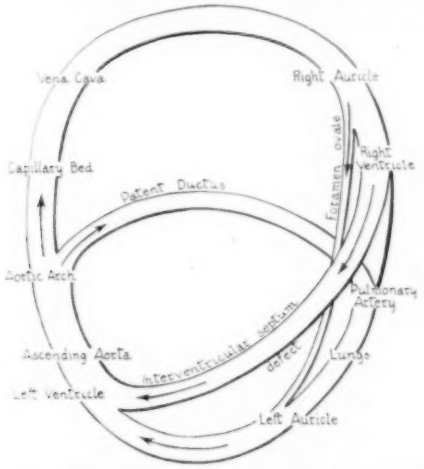

Fig. 9.—Septum defect acting as a veno-arterial fistula, the patent ductus as an arteriovenous fistula; intermittent cyanosis; right auricle and right ventricle dilated and hypertrophied; aorta very wide at origin; greatly diminished after giving off patent ductus.

Case 10 (Fig. 9).[13]—A girl, aged 9 months, was small with an ash gray tint. She was cyanosed during coughing or crying. A loud systolic murmur was heard, and there was increasing dyspnea and weakness.

At necropsy the right auricle and right ventricle were both of abnormally large capacity, their walls hypertrophied. The foramen ovale was patent. The septum of the ventricles was deficient at its upper part, the aortic orifice being so placed as to communicate as directly with the right as with the left ventricle. There was no opening into the pulmonary artery. The aorta was very wide in the ascending portion of its arch, then tapered till it was joined by the ductus arteriosus, when it suddenly became much smaller. The ductus arteriosus was rather wide and its coats were thick, and the right and left pulmonary arteries, both from their direction and their structure, appeared to be branches of the arterial duct coming from the aorta, rather than of the impervious vessel coming from the heart. The lungs presented congestion of their posterior parts and extensive collapse of the lower lobes.

13. Baly: Malformation of the Heart, Tr. Path. Soc. London **10**:90-92, 1859.

CASE 11 (Fig. 10).[14]—A man, aged 31, had been noticed to have a livid appearance from his birth, but he did not appear to have had any symptoms directly referable to the heart until he reached his twenty-seventh year. In November, 1878, he had some swelling of the abdomen, became short of breath, and suffered from slight hemoptysis. July 3, 1882, he was admitted into the Middlesex Hospital suffering with pain in the abdomen and loins. The vessels of the face were markedly injected; the lips were livid, and the finger nails of a slaty bluish color. There was a slight presystolic thrill. The cardiac dulness commenced above at the upper border of the fourth rib, extended to the nipple on the left, and to the border of the sternum on the right. On auscultation the first sound at the apex was very rough; the second sound sharp and ringing. At the base, in the third left interspace close to the sternum, a loud rasping bruit could be heard. This bruit, which was very localized, appeared to follow closely on and partly to conceal the second sound. It was not audible at the aortic cartilage nor at the back. The pulse, which was 96, was of fair volume, compressible and regular. The patient died.

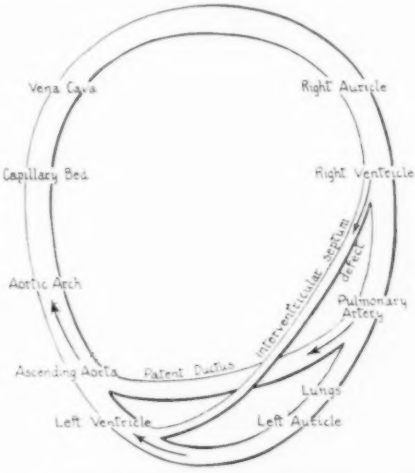

Fig. 10.—Patent septum and patent ductus acting as veno-arterial fistulas; right ventricular wall thicker than left. Cyanosis had been prominent from birth.

At necropsy the peritoneal cavity contained about one-half pint (236.5 c.c.) of serum. The pericardial sac contained 30 ounces (887.2 c.c.) of serum, and the serous surfaces, especially at the base of the heart, were coated with shreds of lymph. The heart weighed 26 ounces (737 gm.); all the cavities were hypertrophied, and the auricles also notably dilated. Coagula occurred in all the chambers. The tricuspid orifice was large, and its valves incompetent; the pulmonary valves were natural and competent, and the infundibulum was natural. The ductus arteriosus, which had been partially laid open in removing the heart, was of large size and freely patent, admitting the tip of the little finger. At its orifice into the aorta there was some calcified atheroma beneath the lining membrane. The aortic valves were competent; the mitral valve

14. Coupland, Sidney: Congenital Malformation of the Heart; Patent Ductus Arteriosus; Defect of Ventricular Septum; Cyanosis; Facial Erysipelas; Pericarditis—Death, M. Times & Gaz., London **2**:501, 1884.

also was competent, but thickened along its border. A rounded aperture large enough to permit the passage of an ordinary cedar pencil occurred in the pars membranacea of the ventricular septum. Whether or not the aorta was narrowed beyond the entrance of the ductus was not observed. The wall of the right ventricle at the base of the heart measured seven-eighths inch (2.1 cm.) in thickness; that of the left six-eighths inch (1.8 cm.). The lungs were highly edematous.

COMMENT

In surveying these remarkable diversions from the normal flow of blood, most instructive information is obtained as to the occurrence and causation of cyanosis. It is evident that cyanosis in all instances was due to the flow of blood through abnormal openings from the venous to the arterial sides of the circulation. These abnormal openings may, therefore, be designated as *veno-arterial* fistulas.[15] In those instances in which the admixture occurred in the opposite direction, i. e., from the arterial to the venous side of the circulation, as in Case 1 and in numerous cases of patent ductus, no cyanosis was recorded. Under such circumstances the abnormal opening serves as an arteriovenous fistula, transmitting only aerated blood. The most intense cyanosis was encountered in Cases 6, 8 and 10, in which the greater part of the blood from the vena cava entirely escaped the lungs and was diverted, unoxygenated, through abnormal openings into the systemic circulation. That cyanosis may be due to the admixture of venous to arterial blood is abundantly proved by these examples, as well as by those cases of pure patent ductus in which the blood flows from the pulmonary artery into the aorta. It is apparent that the authors of books on pediatrics should revise their statements that such an admixture does not explain the cyanosis found in cardiac anomalies.[16]

Abundant evidence is also provided by these circulatory anomalies to substantiate the physiologic principle that the chambers of the heart, as well as the blood vessels, place themselves in harmony with the volume

15. Since the completion of this article and a preceding one on patent ductus arteriosus, my attention has been called to a contribution on the subject by Dr. Maude Abbott, with W. T. Dawson: The Clinical Classification of Congenital Cardiac Disease, Internat. Clin. **4**, Series 34, December, 1924. Dr. Abbott designates the abnormal openings delivering venous blood into the systemic circulation as "venous arterial shunts," and those delivering the arterial blood into the pulmonary circuit as "arterial venous shunts." Although she does not include the patent ductus as a "venous arterial" shunt, it would seem from our studies that it should also be included under this head. Cyanosis in these cases depends, of course, on the amount of unaerated blood that escapes into the systemic circulation, and obviously does not appear unless the diversion of venous blood has assumed considerable proportions.

16. Griffith, J. P. C.: The Diseases of Infants and Children, Philadelphia, W. B. Saunders Company, 1921, p. 126. Graham, E. E.: Diseases of Children, Philadelphia, Lea and Febiger, 1916. Tuttle and Hurford: Diseases of Children, Philadelphia, Lea and Febiger, 1917. Holt and Howland: Diseases of Infancy and Childhood, New York, D. Appleton & Co., 1922.

flow of blood through them. The enlargement of the heart that is found in these cases, restricted to certain chambers only, may be both a dilatation and an hypertrophy, the dilatation in the well functioning organ being solely attributable to the increased minute volume flow through those chambers. The hypertrophy, however, may be attributed to two factors: (1) the increased effort necessary to propel forward an increased minute volume flow of blood, and (2) the assumption by the right heart, in addition to its normal function, of a part of the burden of the left heart in overcoming the systemic peripheral resistance and in supplying the systemic circulation with blood. Whenever abnormal openings, such as the septum defect or the patent ductus, place the right heart in direct communication with the systemic circulation, an increased development of the right ventricle is observed. When, as in Case 9 (Fig. 8), the left heart assumes the full burden of supplying both the systemic and the pulmonary circulations, we have a proportionately greater development of this half of the heart.

The clinical evidence presented by this study of congenital cardiac anomalies is convincing that the heart chambers respond, both by dilatation and by hypertrophy, to an increased volume flow of blood through them, and the deduction is justified that such an increased flow is the most effective physiologic stimulus to cardiac dilatation and hypertrophy.

THE USE OF UREA AS A DIURETIC IN ADVANCED HEART FAILURE *

J. HAMILTON CRAWFORD, M.D.
AND
J. F. McINTOSH, M.D.
NEW YORK

It has been known for a long time that the administration of urea in large doses produces a considerable increase in the volume of urine. Urea was, however, until comparatively recently little utilized for its diuretic properties. This was doubtless due to the prevailing idea that urea retention was the causative factor in uremia. Urea was considered a harmful substance and any measures undertaken to raise its concentration in the body were regarded with disfavor. With the advance of clinical chemistry it soon came to be recognized that although the urea in the blood is raised in uremia it is not the cause of the condition. It became established that if the function of the kidney is good urea can be given to patients for prolonged periods of time with impunity. Even in cases in which kidney disease was known to be present, large doses of urea have been given in order to estimate the functional capacity of the kidney and no untoward results have been observed. Recently, urea has been administered over prolonged periods of time in the treatment of certain cases of nephritic edema in which the blood urea was not raised and very favorable results have been reported.

Urea was first used as a diuretic by Friedrich [1] in 1892. In this investigation he administered urea in edema due to various causes; the series included a few cases of cardiac edema. He gave from 2 to 14 gm. a day and reported very favorable results. Feilchenfeld [2] conducted a similar study with similar results. He stated that the increase in urine volume was proportional to the dose. Strauss [3] gave urea in doses up to 100 gm. a day to twelve patients with cardiac edema following heart disease or arteriosclerosis, with very beneficial results. He commenced with doses of 40 gm. a day and gradually increased it as necessity demanded. In spite of the favorable results reported by the foregoing observers, urea has not come into common use as a diuretic in cardiac edema for reasons we have not been able to ascertain.

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* From the Hospital of the Rockefeller Institute for Medical Research.

1. Friedrich, W.: (Ueber die harntreibende Eigenshaft des Uream) as ureum hugyhajto tulajdonsargarol. Kozleminy u budapeste Magy. kir tud Egyetem gyogyozertani intezelebol, Magy. orv. Arch. **1**:400-415, 1892.

2. Feilchenfeld, J.: Ueber Harnstoff als Diureticum, Therap. d. Gegenw. **59**: 273, 1918.

3. Strauss, H.: Ueber Harnstoff als Diuretikum, **58**:375, 1921.

For our investigation we have selected cases of advanced cardiac decompensation which have responded only partially or not at all to the methods usually employed in the treatment of cardiac edema. The series included four cases of mitral stenosis with auricular fibrillation, two cases of mitral stenosis, one case of aortic disease with auricular fibrillation, and one case of exophthalmic goiter with auricular fibrillation.

The routine treatment of all patients included rest in bed, a salt free diet, thorough digitalization, and restriction of the fluid intake to 1,200 c.c. a day. Four of the patients had also received novasurol, and had been rendered edema free, but after the injections of this drug had been discontinued, the urine volume was subnormal and edema had gradually reappeared. Urea was given with a view to reestablishing a normal water balance by maintaining an adequate urine output, and also, when slight edema had collected, to bring about its removal. The other four patients of our series all exhibited marked edema when urea medication was instituted. These patients had not received a course of novasurol. One case of mitral stenosis with regular rhythm (Case 6) did not receive digitalis as we decided to study the effect of urea without previous digitalization.

In all cases twelve hour specimens of urine were collected, and in these, the volume, the specific gravity and the chlorid excretion were determined. Urea administration was not commenced until the urine volume and chlorid excretion had reached a constant level. In order to study in detail the changes that took place in the urine volume, urea excretion and blood urea, and their time relations, we collected a specimen of urine over a period of seventy-two minutes at frequent intervals and obtained a specimen of blood in the middle of this period. The time of making these tests was varied with relation to the administration of urea in order that its effect might be followed more closely. During these periods no food or fluid was given. The urea in the blood and urine was estimated by the method of Van Slyke and Cullen,[4] the chlorid in the urine by a Volhard titration, and the chlorid in the plasma by the method of Van Slyke.[5]

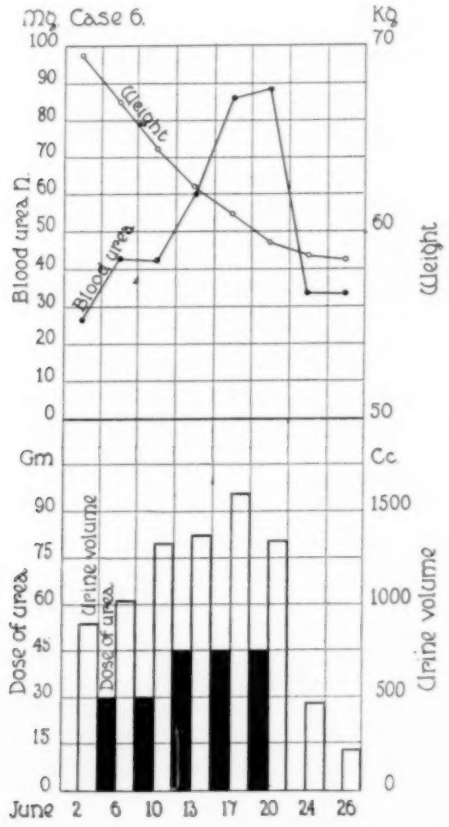
METHOD OF ADMINISTRATION

Urea was given in a small quantity of water a short time after a meal as it had been previously found that gastric disturbance was less likely to take place if it were given at this time. Urea has a peculiar metallic

4. Van Slyke, D. D., and Cullen, G. E.: A Permanent Preparation of Urease and Its Use in the Determination of Urea, J. Biol. Chem. **19**:211, 1914.

5. Van Slyke, D. D.: The Determination of Chlorids in Blood and Tissues, J. Biol. Chem. **58**:523 (Dec.) 1923.

taste and is not pleasant to take. The patients soon become accustomed to it, however. In four of our cases we administered 30 gm. a day in divided doses of 15 gm. One dose was given at 8:30 a. m. and the other at 4 p. m. In the other four cases, we studied the effect of increasing the quantity of urea. At first 30 gm. a day was given. This



Effect of urea administration on urine volume, weight and blood urea. The patient had received one-third the daily dose of urea at 8:30 and 12:30; specimens of blood were obtained at 2 p. m.

was then increased to 45 gm. a day, and in two cases it was further increased to 60 gm. a day. In three of the cases these increases took place at weekly intervals. The urea was given in three equal doses at 8:30 a. m., 12:30 and 6 p. m.

OBSERVATIONS

Urine Volume.—The response of the cases with edema to urea feeding was prompt and, as a rule, very efficient (accompanying chart). In seven cases of the series, oliguria was present before treatment was instituted. Their average daily output during the control period varied from 340 to 776 c.c. After 30 gm. of urea a day, the average daily urine volume for each case varied, as a rule, between 900 and 1,300 c.c. When the amount was increased to 45 gm. a day, the average daily output increased to 1,400 and 1,500 c.c. A dose of 60 gm. daily was followed by a further slight increase (Tables 1, 2 and 3). The maximum response to a particular dose did not occur until the second or even the third day that the urea was given. The daily output then remained at this level as long as this particular dosage was continued. After urea administration was discontinued, a diuretic action was still distinguishable during the first twenty-four hour period thereafter.

The time relations between the dosage and the diuretic response can be still further analyzed by studying the rate of excretion during the seventy-two minute periods. From these data it may be seen (Table 2) that an increased urine volume was already established within three hours after the first dose was given. After the urea feeding had been discontinued, the duration of its diuretic action depended to some extent on the dosage. When 30 gm. a day was given, its effect had practically passed off within fifteen to seventeen hours. But with larger doses, its influence was still apparent at this time (Table 1).

Weight of Patient.—Patients with edema lost weight corresponding to the diuresis that took place. This loss of weight was gradual and continuous (chart). Patient 7, who did not have oliguria during the control period, is particularly noteworthy. In this case the diuresis was much greater than in any of the others and the weight loss much more rapid, amounting to 8 kg. in the first five days; this is to be contrasted with a loss of 1.6 kg. in the preceding control period of five days. In some cases continuance of urea feeding was necessary to prevent accumulation of edema, for when it was stopped the patient's weight tended to increase, until visible edema reappeared (Tables 1, 2 and 3).

Clinical Condition.—Corresponding to the weight loss, one noted a disappearance of edema, and of transudates from the body cavities. It was possible to clear all the patients of excess fluid in this way, with the exception of Patient 8. At the same time definite subjective improvement occurred. The most striking clinical improvement occurred in Case 7, in which rapid subjective improvement occurred simultaneously with the loss of edema. Patients 4, 5 and 6 also experienced very definite symptomatic relief which other measures had failed to give.

This relief was associated with a decrease in weight. Cases 1 and 2 were free from edema at the beginning of treatment, and there was no definite symptomatic improvement due to urea. Case 3 improved subjectively when urea medication was commenced, although edema was absent and weight loss did not occur. The value of urea in the last three cases was in maintaining a normal water balance. When the urine volume diminished consequent on the withdrawal of urea, edema tended to recur, with its concomitant symptoms. When the administration of urea was resumed, symptoms and edema were again controlled.

In Case 8, urea administration did not result in clinical improvement. This was a case of mitral stenosis with normal rhythm in which there was considerable ascites, and slight edema of the legs. The patient reacted to a slight extent to digitalis, but this effect was transitory. The dose of urea was gradually increased up to 60 gm. a day, each increase causing a definite rise in urine volume. Nevertheless, the clinical condition did not improve. After nineteen days of treatment the patient had lost 4.5 kg. in weight. The patient began to complain of nausea and on the last day of the course vomiting occurred. The blood urea nitrogen was high after the midday dose, 74.9 mg. per hundred cubic centimeters. Accordingly, urea was discontinued. The urine volume immediately fell and the general state of the patient became worse. It then became apparent that urea had been beneficial to the extent of postponing the unfavorable clinical course that subsequently developed.

Evidence of Intolerance.—When the dose was taken on an empty stomach, vomiting occasionally occurred immediately after, but this symptom gave little trouble when the urea was given after meals. In three of the caes (Cases 5, 7 and 8) the prolonged administration of urea in large doses gave rise to untoward symptoms. These manifestations consisted in loss of appetite, nausea and sometimes vomiting, and in one instance, a feeling of weakness and lassitude. In Cases 7 and 8 they were sufficiently urgent to call for the discontinuance of urea administration. In these cases the blood urea nitrogen, estimated in the afternoon after the second daily dose, was 89.0 mg. and 74.9 mg. per hundred cubic centimeters, respectively. These figures are practically identical with those found by Hewlett, Gilbert and Wickett [6] in experiments designed to investigate the toxicity of urea. In normal subjects they found that similar symptoms occurred when the blood urea reached values above 150 mg. per hundred cubic centimeters (equivalent to 70 mg. of urea nitrogen per hundred cubic centimeters). In our cases the symptoms disappeared, as a rule, within twenty-four hours after urea

6. Hewlett, A. W.; Gilbert, Q. O., and Wickett, A. D.: The Toxic Effects of Urea on Normal Individuals, Arch. Int. Med. **18**:636 (Nov.) 1916.

TABLE 1.—*Effect of Urea on Urine Volume and Body Weight; the Blood Urea Nitrogen from Fifteen to Seventeen Hours After Urea Was Given, with the Rate of Urea and Urine Excretion at This Time*

Case	Disease	Age	Dose of Urea, Gm.	Number of Days on Which Urea Was Given	Number of Days Without Urea	Weight at End of Period, Kg.	Average Daily Urine Volume, C.c.	Chlorid Excretion in 24 Hours as Sodium Chlorid, Gm.	Blood Urea Nitrogen per 100 C.c. Mg.	Rate of Urine Excretion per 24 Hours Calculated from 72 Minute Period, C.c.	Urea Nitrogen Excretion Rate per 24 Hours Calculated from 72 Minute Period, Gm.	Urea Excretion Index, $\frac{10\ D}{B\sqrt{VW}}$	Remarks
1	Aortic disease (syphilitic); auricular fibrillation	47	..	..	..	90.6	518	1.63	13.1	500	4.64	54.2	Before urea
			30	7	..	87.6	1,009	2.47	.....	.....	.....	.....	
			..	..	4	86.4	848	1.08	14.7	500	5.70	56.0	Novasurol, 1 c.c. on second day*
			30	8	..	84.3	1,280	1.39	.....	.....	.....	.....	
			45	5	..	86.0	1,544	0.77	25.8	1,460	18.20	63.2	
			45	10	..	85.2	1,370	0.86	35.8	1,680	24.32	56.7	
			..	..	1	85.5	875	1.65	.....	.....	.....	.....	
			60	14	..	85.5	1,558	0.62	37.6	1,520	23.70	55.3	
2	Mitral stenosis; auricular fibrillation	31	..	..	..	53.4	585	2.70	10.5	340	1.64	56.6	Before urea
			30	6	..	53.8	980	3.09	15.8	720	9.34	95.2	
			30	4	..	54.0	931	2.15	.....	.....	.....	.....	
			..	..	3	54.4	743	3.96	10.6	888	5.62	76.9	
			30	11	..	53.9	985	3.15	18.8	696	9.68	84.3	
3	Mitral stenosis; auricular fibrillation	37	..	..	..	50.8	385	0.08	11.4	656	1.97	29.9	Before urea
			30	27	..	51.4	1,012	0.27	22.7	648	11.16	85.1	
			30	4	..	52.5	914	0.21	25.5	760	10.60	66.8	
4	Mitral stenosis; auricular fibrillation	20	..	..	..	41.1	447	1.70	14.3	460	4.16	69.2	Before urea
			30	6	..	38.2	863	2.37	24.7	780	4.64	54.8	Novasurol, 2 c.c. on fifth day*
			30	12	..	38.3	831	2.52	28.1	544	9.05	71.5	
			..	..	4	38.0	573	0.90	10.6	780	9.64	84.0	
			..	..	4	38.3	508	2.24	8.2	700	4.57	109.1	
			30	11	..	37.2	845	2.48	.....	.....	.....	.....	Novasurol, 2 c.c. on fourth day*
5	Exophthalmic goiter; auricular fibrillation; ascites; slight edema of the legs	46	..	..	..	44.9	688	2.86	15.5	1,000	4.72	47.0	Before urea
			30	4	..	44.0	1,423	3.65	19.4	900	8.44	70.9	
			30	9	..	41.8	1,199	2.69	.....	.....	.....	.....	
			..	..	6	43.8	640	1.28	.....	.....	.....	.....	
			30	5	..	43.1	1,413	3.42	24.3	880	5.19	35.6	
			30	5	..	42.5	1,298	3.47	.....	.....	.....	.....	
			..	..	2	42.4	964	2.12	6.6	560	2.31	31.5	
			..	..	5	44.1	940	1.81	.....	.....	.....	.....	
			30	22	..	42.7	1,339	3.89	.....	.....	.....	.....	
			45	15	..	42.2	1,556	1.70	36.6	560	6.62	37.5	
			..	..	5	42.0	1,125	0.51	.....	.....	.....	.....	

* Days on which novasurol was effective are not included.

was discontinued. In Case 6, however, they persisted to a lessened extent for four days thereafter. This case had a coexistent nephritis. After urea had been discontinued on account of symptoms of intolerance, it was always possible after a short interval to resume treatment, with a smaller dose, without the production of untoward symptoms.

Most of the patients, although not all, complained of thirst while under urea medication; however, this was never severe enough to interfere with the continuation of treatment. Headache was an occasional but never a severe cause of complaint. It is not certain that any of these symptoms were entirely due to urea administration; the benefits derived from the urea were marked and in our judgment far outweighed the discomfort entailed.

CHANGES IN URINARY CONSTITUENTS

Urea.—A study of the rate of urea excretion shows that this ran parallel with the water excretion. The amount gradually increased for two or three days after treatment was begun and thereafter remained practically constant as long as the dosage remained constant, irrespective of the time it was continued. With larger doses the amount of urea excreted was increased. Cessation of treatment rapidly reduced the output of urea to its previous level. The increased excretion of urea began within from one and one-half to three hours after its administration (Table 2). Later in the day, after a second dose of urea had been given, the amount excreted was still greater (Table 3). On the following morning much less urea was excreted, the amount depending largely on the dosage. With the smaller doses, the quantity of urea in the urine seventeen hours after the last dose was only raised to a slight extent over the amount excreted before urea was given. With the larger doses, however, an increased output was still maintained at this time (Table 1).

Chlorid.—As a rule more chlorid is excreted during urea administration than during the control period. The changes in amount are slight, however, and show very little regularity.

Abnormal Urinary Changes.—We have seen no evidence of kidney irritation as shown by the presence of albumin, red blood cells or casts. When these were present before treatment was begun they became progressively reduced in amount and in almost every case disappeared.

CHANGES IN THE BLOOD

Urea.—The blood urea nitrogen was increased by taking urea. The extent of the increase varied with the dose (chart). When a certain amount of urea was given on succeeding days in divided doses, the hours of administration being the same on these days, the concentration

TABLE 2.—*Effect of Urea on Urine Volume and Body Weight; the Blood Urea Nitrogen Shortly After the Morning Dose (One and a Half to Three Hours) During Time Urea Was Given Twice Daily, with the Rate of Urea and Urine Excretion at This Time*

Case	Disease	Age	Dose of Urea, Gm.	Number of Days on Which Urea Was Given	Number of Days Without Urea	Weight at End of Period, Kg.	Average Daily Urine Volume, C.c.	Chlorid Excretion in 24 Hours as Sodium Chlorid, Gm.	Blood Urea Nitrogen per 100 C.c., Mg.	Rate of Urine Excretion per 24 Hours Calculated from 72 Minute Period, C.c.	Urea Nitrogen Excretion Rate per 24 Hours Calculated from 72 Minute Period, Gm.	Urea Excretion Index, $\frac{10\ D}{B\sqrt{VW}}$	Remarks
2	Mitral stenosis; auricular fibrillation	31	..	..	..	53.4	585	2.70	10.5	340	1.64	36.6	Before urea
			30	32	..	54.9	837	2.73	24.4	1,336	23.50	114.0	Received 15 gm. of urea 1½ hours before bleeding
			..	..	5	54.6	461	1.15	10.4	576	5.24	90.8	
			30	10	..	55.4	1,017	2.21	25.5	1,392	23.45	106.6	
3	Mitral stenosis; auricular fibrillation	37	..	..	..	50.8	385	0.08	11.4	656	1.97	29.9	Before urea
			30	2	..	51.0	940	0.50	25.1	1,056	16.47	89.5	Received 15 gm. of urea 3 hours before bleeding
			30	23	..	52.3	1,043	0.17	36.8	864	16.94	69.4	

of urea in the blood *at a given time* rose for a few days, until a certain height was reached. It continued at about this level however long this dosage was continued. As soon as the administration of urea was discontinued, the amount of urea nitrogen in the blood rapidly fell to its previous level. The volume of urine and urea excretion reflected the concentration of the urea in the blood. Seventeen hours after the last dose the urea in the blood was still raised—the height varying with the amount administered—but the increase was not great (Table 1). The urea given must have been rapidly absorbed for the amount of urea nitrogen in the blood increased within three hours (Table 2).

Plasma Chlorid.—The chlorid in the plasma remained practically unaltered.

INDEX OF UREA EXCRETION

A study of kidney function by the urea excretion index of Austin, Stillman and Van Slyke[7] is of considerable interest. These authors have made a study of the changes in the rate of urea excretion with varying concentrations of urea in the blood and with varying urine volumes. They have expressed the relationships between these factors in the following equation: $K = \frac{10\ D}{B\sqrt{VW}}$, in which D represents urea output (as grams per twenty-four hours), B the blood urea (as grams per liter), V the volume output (as liters per twenty-four hours), W the body weight (as kilograms), and K the excretory constant. For the normal subject K varies between 45 and 105. In kidney disease, values varying between 3 and the normal values have been observed.

With the exception of Case 1, all cases in the series fell below the minimum normal value of 45 on some occasion. In Case 6, this subnormal value was chiefly due to a chronic nephritis. In Cases 2, 3, and 6 there was a marked tendency of the index to rise as treatment was continued. This rise was coincident with a marked improvement in the clinical condition. Two cases in the series (Cases 7 and 8) showed low indexes during treatment. Case 8 showed a primary rise of the index with a subsequent fall to a still lower level. It will be recalled that this case did not improve with treatment. Case 7, however, showed a marked improvement, and finally the patient went home with compensation reestablished. The two markedly subnormal indexes found in his case are unexplained. There was no evidence of nephritis.

The general tendency of the index to rise during urea feeding does not occur in normal subjects.[7] It would seem to reflect a general improvement in the clinical condition of the cases under urea treatment. It gives further evidence against the possibility of kidney damage due to urea.

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TABLE 3.—*Effect of Urea on Urine Volume and Body Weight; the Blood Urea Nitrogen One and a Half Hours After the Second Dose During Time Urea Was Being Given Thrice Daily, with the Rate of Urea and Urine Excretion at This Time*

Case	Disease	Age	Dose of Urea, Gm.	Number of Days on Which Urea Was Given	Number of Days Without Urea	Weight at End of Period, Kg.	Average Daily Urine Volume, C.c.	Chlorid Excretion in 24 Hours as Sodium Chlorid, Gm.	Blood Urea Nitrogen per 100 C.c. Mg.	Rate of Urine Excretion per 24 Hours Calculated from 72 Minute Period, C.c.	Urea Nitrogen Excretion Rate per 24 Hours Calculated from 72 Minute Period, Gm.	Urea Excretion Index, $\frac{10\,D}{B\sqrt{VW}}$	Remarks
6	Mitral stenosis; aortic disease (rheumatic); chronic nephritis; ascites; edema of the legs	47	..	..	..	69.8	776	2.20	24.1	1,080	3.14	16.4	Blood pressure 180 systolic, 90 diastolic
			30	4	..	67.1	1,129	2.30	43.2	1,700	10.67	24.7	
			30	4	..	64.7	1,042	2.39	43.2	1,480	.....	....	
			45	3	..	62.4	1,458	2.47	60.9	1,900	10.30	16.0	
			45	4	..	61.0	1,416	1.77	86.6	1,120	9.16	13.0	
			45	3	..	59.7	1,269	....	89.0	1,720	14.15	15.8	
			..	..	4	58.9	555	....	34.3	700	6.09	27.7	
			..	..	2	58.6	226	....	34.9	560	8.89	44.4	
7	Chronic myocarditis; auricular fibrillation; edema of the legs	60	..	..	..	57.6	1,490	12.84	16.6	840	6.57	62.8	
			30	5	..	49.6	2,338	9.89	39.8	2,500	.....	....	
			45	3	..	48.1	1,569	3.50	53.8	2,100	14.68	37.0	
			45	4	..	47.2	1,285	1.00	60.0	1,900	10.05	17.7	
			..	..	2	47.5	726	....	15.8	960	6.25	58.7	
			..	..	5	48.7	980	1.06	21.0	1,500	3.82	21.6	
8	Mitral stenosis; ascites; edema of the legs	47	..	..	..	55.6	344	0.71	21.4	680	6.14	48.6	
			..	..	..	53.6	615	1.00	16.9	400	6.69	87.4	Nine days after previous determination
			30	2	..	53.5	575	0.33	54.0	880	.....	....	
			30	3	..	53.4	692	0.46	41.1	1,060	17.99	59.4	
			45	4	..	53.1	900	0.52	56.9	1,600	14.41	28.6	
			45	3	..	52.9	961	0.89	57.4	3,160	18.99	25.9	
			60	4	..	51.9	1,050	....	78.2	2,500	19.99	22.6	
			60	3	..	51.1	1,115	....	74.9	2,520	19.94	23.4	

COMMENT

Many of the most troublesome symptoms of cardiac decompensation are due to the accumulation of fluid in the tissues, in body cavities and in parenchymatous organs. The most important causal factor is certainly the failure of the efficiency of the myocardium, and it is to the amelioration of this that our first efforts must be directed. But frequently in the more advanced cases of heart disease, the maximum efficiency that can be obtained by treatment of the heart per se does not suffice to remove edema nor to maintain the patient free from symptoms. In the cases treated with urea a marked improvement in the clinical condition of the patient took place which could be assigned to the action of urea. The treatment succeeded in maintaining an adequate urine output and also in removing edema. As soon as the administration of urea was stopped, the urinary output immediately fell and the clinical condition became worse. When treatment was resumed an improvement again took place. We have studied some of our cases for months and have found that a particular dose will give the same daily urinary output with only slight variations throughout the period investigated. In these cases it has seemed that the maintenance of an adequate water excretion has been instrumental not only in preventing symptoms but also in avoiding a relapse. We have also prescribed its use at home by patients who have been discharged from the hospital after treatment for decompensation. Here again careful treatment of the cardiac condition is of prime importance, but it seems that urea has been of great assistance in keeping these patients comfortable through the maintenance of a normal water balance.

Urea is rapidly absorbed and an increase immediately occurs in the height of the urea in the blood. The mean level of the urea in the blood is dependent on the dosage, and the relationship between them is fairly constant in any particular case. The amount of urea excreted depends on the blood urea, so that with constant urea administration a state of equilibrium is reached between the intake and the output. During urea diuresis the excretion of water runs more or less parallel with that of urea, so that urine volume reflects the concentration of urea in the blood. Undoubtedly the explanation of the diuresis is that the excess of urea circulating in the blood is excreted by the kidney, and during the process carries with it a considerable amount of water.

CONCLUSIONS

1. Urea was given in doses of from 30 to 60 gm. a day to eight patients with advanced heart failure and was followed by a marked increase in urine volume. The drug was particularly useful in cases in which an adequate water excretion was not maintained after the

edema fluid had been removed by other measures. In some cases it relieved the edema when other remedies had failed.

2. The increase in urine output varied with the dose and followed closely the curve of urea excretion. With continuous administration the daily urine volume was maintained at an almost constant level. The response after administration was rapid, but the effect passed off in a short time unless the dose was repeated.

3. The changes in urine volume and in urea excretion were dependent on the concentration of the urea in the blood.

4. In several of the cases there was a subnormal index of urea excretion which seemed ascribable to advanced heart failure. The index tended to improve with urea administration, along with a general improvement in the clinical condition.

5. Toxic symptoms of any significance did not take place.

6. From these observations we would suggest that urea is a useful diuretic in cases of heart failure with edema in which treatment of the cardiac condition has failed to remove the edema or maintain an adequate water excretion.

DISEASES OF THE LIVER

IV. FUNCTIONAL TESTS IN CASES OF CARCINOMA OF THE LIVER AND BILIARY TRACT *

CARL H. GREENE, M.D.
CHARLES S. McVICAR, M.D.
WALTMAN WALTERS, M.D.
AND
LEONARD G. ROWNTREE, M.D.
ROCHESTER, MINN.

Success in the surgical treatment of carcinoma is attained only when complete removal of the primary malignant lesion is possible. Metastatic dissemination precludes other than palliative measures.

Early diagnosis of intra-abdominal malignant disease, especially of carcinoma involving the hollow viscera, has been greatly facilitated by the roentgen ray. Demonstration of a significant filling defect, especially in the stomach or large bowel, as a rule is possible long before the malignant growth causing such a defect has produced a palpable or visible tumor, and frequently before marked cachexia has developed. The lay conception of cancer as a painful, debilitating, wasting disease is based on clinical pictures of advanced and often incurable malignant disease. The onset in any adult of dyspepsia which resists the usual methods of relief, and remains persistent or progresses, should at least evoke a suspicion of gastric carcinoma until another explanation is found. Similarly, marked or prolonged changes in the activity of the bowels in a person near or past middle life is always suggestive of malignancy of the large bowel. In such a case the probability of carcinoma must be kept in mind until another cause is shown. The suspicion of gastro-intestinal carcinoma must be aroused earlier in the minds of laymen and physicians, and propaganda to correct the preroentgen-ray conception of cancer is needed. Assuming early diagnosis and the correspondingly more reasonable chance that the malignant lesion is operable, prognosis will still depend on the presence or absence of metastasis. Palpable implants on the rectal shelf, demonstrable ascites, the rare but deadly button-like infiltration of the umbilicus, roentgenologic evidence of malignant infiltration of the lung, the presence of a "sentinel" gland in the supraclavicular region, or of grossly palpable nodules on the surface of the liver, are all contraindications to radical surgical treatment. Even in the absence of such evidence, laparotomy

* From the Divisions of Medicine and Surgery, Mayo Clinic, and The Mayo Foundation.

may reveal metastatic nodules in the liver. Any laboratory test, therefore, which may be depended on to disclose the existence of malignant infiltration of the liver, either primary or metastatic, will be of definite clinical value.

Rowntree, Hurwitz and Bloomfield,[1] and more recently Rosenthal,[2] Ottenberg, Rosenfeld and Goldsmith,[3] and Gonzalez and Karr [4] have reported studies of the phenoltetrachlorphthalein test in cases of carcinoma involving the liver, and have suggested that the test is of particular value in such cases. Their studies, however, were not sufficiently extensive to determine the exact clinical value of the test.

In previous papers,[5] we have reported a series of tests for hepatic function in patients with obstructive jaundice and in animals with experimentally induced obstruction of the common bile duct. Bilirubinemia and marked retention of phenoltetrachlorphthalein are both characteristically present. Particularly striking was the parallelism between the degree of jaundice and the extent of the dye retention. Included in the latter report was a series of cases of obstruction secondary to tumor of the head of the pancreas, presumably carcinomatous in nature. In these cases, however, the jaundice completely dominated both the clinical picture and the laboratory findings.

We shall now present the results of a study of these tests in three groups of cases: Group 1, fourteen cases of metastatic carcinoma of the liver associated with frank jaundice; Group 2, eighteen cases of metastatic carcinoma of the liver without jaundice, and Group 3, seventeen cases of intra-abdominal carcinoma without demonstrable involvement of the liver.

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GROUP I. METASTATIC CARCINOMA OF THE LIVER ASSOCIATED WITH FRANK JAUNDICE

In the presence of demonstrated carcinoma elsewhere, jaundice, when present, ordinarily constitutes incontrovertible evidence of involvement of the liver or the biliary tract. Usually it is evidence of obstruction of the extrahepatic biliary passages, although not necessarily so. Table 1 shows the findings with the different tests for hepatic efficiency in a series of fourteen cases of malignant disease of the liver associated with frank jaundice.

Only those positive findings on which conclusions are based are included in the cases reported. The phenoltetrachlorphthalein reading is recorded as for the end of the first hour only. Complete findings are included in the tables. Details of the surgical procedures are not given since the patients were regularly prepared according to Walters' method.

CASE 1 (Table 1).—*Carcinoma of the tail of the pancreas with metastasis to the liver, and jaundice without obstruction to the biliary passages.*

A man, aged 58, came to the Mayo Clinic, Nov. 17, 1924, because of marked loss of weight and extreme jaundice of four weeks' duration. In August, 1924, he had first noticed a dull pain localized in the right epigastrium, which was accompanied by nausea and relieved by vomiting. He had had two or three attacks of pain, which was never severe or colicky. Jaundice had developed four weeks before admission; it fluctuated in intensity at the onset. The stools were light and the urine dark. Moderate pruritus was present. Jaundice, constipation, weakness, malaise, dyspnea, anorexia and nausea were increasingly evident. During this period he lost 52 pounds (23.6 kg.) although he still weighed 254 pounds (115.2 kg.).

The liver was enlarged and tender, extending 6 cm. below the costal margin. The surface was smooth and the edge sharp. Fluoroscopic examination of the stomach showed an extragastric mass. No glandular enlargement was found. The urine contained much bile and a trace of albumin. The serum bilirubin was 71.3 mg. Retention of phenoltetrachlorphthalein was extreme, the one hour reading being 51 per cent. The patient failed rapidly and progressively. Hematemesis appeared November 25, and he became comatose. The blood urea became elevated to 68 mg. two days before death, November 27.

At necropsy, carcinoma of the tail of the pancreas with extensive metastatic involvement of the liver, kidneys and lungs was found, but curiously enough there was no mechanical obstruction to the extrahepatic biliary passages. This case is of unusual interest in showing the possibility of the development of an extreme degree of jaundice in the absence of demonstrable obstruction to the extrahepatic biliary passages.

CASE 3 (Table 1).—*Primary carcinoma of the bile ducts with involvement of the liver and obstructive jaundice.*

A man, aged 60, came to the Mayo Clinic, May 6, 1924, because of attacks of abdominal pain and of jaundice of one month's duration. For the preceding year he had suffered from epigastric fulness and distress after meals. One month before admission and following a rough automobile ride, he had an attack of severe, disabling pain in the upper part of the abdomen, accompanied by nausea and followed by soreness along the right costal margin. He subsequently had four or five similar attacks, each of approximately five minutes' duration. Jaundice appeared after the second of these attacks.

He had lost 15 pounds (6.8 kg.) during the three weeks preceding his admission. Marked jaundice was present. The liver edge was palpable 4 cm. below the costal

margin and a rounded mass, approximately 10 cm. in diameter, was felt below the liver edge. There was a suggestion of free fluid in the abdomen. Urinalysis revealed bile 3, but no albumin or casts. The serum bilirubin was 43.2 mg. There was marked retention of phenoltetrachlorphthalein, with a reading of 33 per cent. During the twelve days the patient was in the hospital, the jaundice decreased from 4 to 2. Bile appeared in the stools in small amounts at this time. June 11 the serum bilirubin was 26.5 mg. The phenoltetrachlorphthalein reading was 26 per cent.

At operation, June 18, an inoperable carcinoma, evidently primary in the bile ducts, was encountered. There was marked involvement of the liver and of the hepatic, common and cystic ducts. The gallbladder was large and distended, but was not opened.

The similarity between the history and the symptoms in this case to those in cases of stone is marked, and a clinical diagnosis of obstructive jaundice due to stone in the common duct was made. While the patient was under observation, the jaundice decreased and a small amount of bile was present in the stool. A similar decrease in the degree of jaundice while the patient was under observation was seen in Case 5. It is evident that such changes cannot be interpreted as pathognomonic of obstruction due to stone. This case is also of interest because of the similarity between the fall in the serum bilirubin and the degree of dye retention which accompanied the temporary appearance of bile in the stool on the one hand, and the improvement seen in cases of obstructive jaundice following the operative relief of the obstruction on the other. This illustrates the preponderant effect of the jaundice and bile retention on the functional findings in the cases of carcinoma with obstruction to the biliary passages.

CASE 6 (Table 1).—*Carcinoma of the head of the pancreas with involvement of the liver and obstructive jaundice.*

A man, aged 34, was admitted to the Mayo Clinic, August 6, 1924, because of painless jaundice of ten weeks' duration. There was a history of a questionable chancre fifteen years before, but the Wassermann reaction was negative. For three months previous to the onset of the jaundice, the patient had been drinking excessively. Two days before, he noticed that the urine was dark and the stools light. The stools later became clay colored. He consulted a physician who gave him three injections of neoarsphenamin without the jaundice being affected. Bile was obtained by duodenal drainage at that time.

The patient's weight on admission was 116 pounds (52.6 kg.), representing a loss of 30 pounds (13.6 kg.) in three months, and extreme jaundice was present. There were no palpable lymph nodes. The liver was enlarged, the edge being palpable at the level of the umbilicus; the edge was sharp and the surface smooth. No nodules were felt. The gallbladder and spleen were not palpable. The urine contained albumin, casts and bile. The stools were clay colored, and tests for bile were negative. The guaiac test for blood in the stools was occasionally positive. The serum bilirubin was 36.4 and there was marked retention of phenoltetrachlorphthalein, the reading being 24 per cent. Duodenal drainage was attempted on three occasions; there was no return of bile though traces of blood were obtained.

The patient was kept under observation for ten days, during which time the pulse and temperature were normal. Thereafter the course was strikingly febrile, with repeated chills and a temperature varying between 102 and 104 degrees. The anemia increased rapidly, the hemoglobin falling to 35 per cent. A transfusion was given without benefit. The stool remained acholic, but the serum bilirubin

TABLE 1.—*Carcinoma*

Case	Date	Age, Years	Sex	Edge of Liver Palpable, Cm.	Jaundice	Duration of Jaundice, Months	Urine: Specific Gravity	Urine: Albumin	Urine: Casts	Urine: Bile Pigments	Blood Count: Hemoglobin, per Cent.	Blood Count: Erythrocytes, Millions	Blood Count: Leukocytes	Phenolsulphonephthalein, per Cent.	Blood Nitrogen Partition, Mg. per Cent.: Nonprotein Nitrogen	Blood Nitrogen Partition, Mg. per Cent.: Urea	Blood Nitrogen Partition, Mg. per Cent.: Uric Acid	Blood Nitrogen Partition, Mg. per Cent.: Creatinin	Blood Nitrogen Partition, Mg. per Cent.: Amino-Acid Nitrogen
							Diagnosis Confirmed Pathologically												
1	11/17/24	53	M	6	4	1	1.012	1	0	4	65	4.03	16,200	..	..	24	2.6	2.0	..
2	12/ 6/24	61	M	*	4	1	1.023	3	0	...	70	4.23	15,000	..	..	22	1.7	...	...
3	5/30/24	60	M	4	4	1	1.012	0	0	3	71	4.41	6,500	..	22	23	2.2	1.6	6.0
4	6/11/24	..	..	....	2	....	.....	...	...	...	..	....	.....	..	23	54	...	1.1	5.1
	6/22/23	49	F	0	2	1	1.017	3	1	2	75	4.50	10,700	30	..	16	4.1	2.7	6.7
5	11/26/23	59	F	4	4	2 wk.	1.010	2	0	+	73	4.80	26,200	45	..	28	3.1	1.4	...
	12/ 5/23	..	..	4	2	...	1.006	1	0	....	..	....	14,800	60	...	14	...	...	...
6	8/ 9/24	36	M	15	4	3	1.016	2	1	3	60	3.70	8,250	27	26	20	1.6	1.5	4.8
7	6/23/23	67	M	*	2	18	1.021	1	0	...	85	....	10,900	..	..	32	3.5	2.2	6.1
							No Pathologic Diagnosis Made												
8	9/29/24	70	F	*	4	1	1.007	1	1	3	73	4.87	14,700	..	27	33	2.1	1.8	6.1
9	10/30/24	61	M	6	3	2 wk.	1.025	1	0	3	73	4.59	7,000	..	29	13	1.7	1.7	5.7
10	8/15/24	63	M	†	2	1	1.025	1	0	1	83	4.14	8,600	80	32	32	2.6	1.8	5.2
11	3/ 5/25	46	M	5*	4	6	1.016	1	0	...	80	....	.....	80	20	15	...	...	...
12	3/31/24	74	M	5*	4	1	1.040	3	3	3	62	....	3,800	..	29	24	...	1.4	5.4
13	3/ 5/24	60	F	8*	4	4	1.016	...	...	...	58	3.24	9,800	80	24	11	...	...	...
14	5/26/24	45	M	2*	3	2	1.025	2	...	4	90	4.20	5,800	20	..	26	...	2.5	5.1

* Large and nodular. † Ascites prevented palpation.

TABLE 2.—*Carcinoma*

Case	Date	Age, Years	Sex	Edge of Liver Palpable, Cm.	Jaundice	Urine: Specific Gravity	Urine: Albumin	Urine: Bile Pigments	Blood Count: Hemoglobin, per Cent.	Blood Count: Erythrocytes, Millions	Blood Count: Leukocytes	Phenolsulphonephthalein, per Cent.	Blood Nitrogen Partition, Mg. per Cent.: Nonprotein Nitrogen	Blood Nitrogen Partition, Mg. per Cent.: Urea	Blood Nitrogen Partition, Mg. per Cent.: Uric Acid	Blood Nitrogen Partition, Mg. per Cent.: Creatinin	Blood Nitrogen Partition, Mg. per Cent.: Amino-Acid Nitrogen
						Diagnosis Confirmed Pathologically											
15	6/11/24	73	M	0	0	1.028	1	..	72	4.08	6,000	..	33	46	2.4	1.7	5.1
16	7/ 2/24	46	F	8	0	1.022	0	+	65	3.80	19,200	..	27	35	3.0	2.0	5.4
17	12/ 6/24	42	F	*	0	1.015	4	0	73	4.52	7,100	..	28	22	3.3	1.3	4.9
18	8/ 9/24	72	M	*	±	1.019	1	..	80	4.08	22,300	15	276	406	6.5	6.3	8.7
19	5/ 3/24	56	M	0	0	1.010	1	0	67	....	12,700	45	29	28	2.8	1.6	6.9
20	3/ 5/25	55	M	*	0	1.018	0	0	19	1.61	6,900	..	..	62	...	1.4	...
21	12/ 8/24	48	M	0	0	1.027	0	0	43	2.91	8,900	..	35	27	...	1.3	5.7
22	12/ 8/24	60	M	0	0	1.027	0	0	73	4.44	8,400	..	33	12	3.4	1.3	6.5
23	1/12/25	30	M	0	0	1.028	2	0	68	4.31	10,900	..	..	25	2.5	1.1	...
24	10/24/24	36	M	0	0	1.027	0	0	64	4.56	21,000	..	29	27	3.0	1.6	6.3
25	2/ 9/25	70	M	0	0	1.025	0	0	25	2.67	5,700	..	..	42	3.3	1.2	...
						No Pathologic Diagnosis Made											
26	11/31/23	70	M	*	0	1.026	1	..	80	....	9,800	..	..	..	...	...	...
27	2/28/24	60	M	†	±	1.022	1	0	80	4.15	7,500	55	34	36	3.0	1.8	...
	3/13/24	..	..	†	±	1.015	0	..	..	....	.....	..	35	44	...	...	...
28	3/ 4/24	58	F	15	±	1.017	0	..	77	4.27	6,500	..	33	31	...	...	...
29	3/ 9/25	73	M	†	0	1.027	0	0	70	4.32	7,800	..	..	28	2.9	2.1	...
30	11/14/24	45	F	*	0	1.010	1	0	43	3.54	15,800	45	..	16	2.7	1.6	...
31	11/28/24	39	M	*	0	1.027	1	..	60	3.62	17,400	65	31	32	2.4	1.4	5.1
32	3/ 9/25	64	M	*	0	1.024	1	..	51	4.14	6,800	..	52	52	3.0	2.0	...

* Liver enlarged and nodular. † Liver palpable.

Liver with Jaundice

Bile Pigments (Serum)			Coagulation Time		Fructose Tolerance Blood Sugar, Mg. per Cent.				Phenoltetrachlorphthalein Dye in Serum, per Cent.				
Bile Index	Serum Bilirubin, Mg. per Cent.	Van den Bergh Direct Reaction	Minutes	Seconds	Normal	1 Hour	2 Hours	Change	15 Minutes	1 Hour	2 Hours	Dye in Urine, Mg.	Remarks
					Diagnosis	Confirmed	Pathologically						
...	71.3	+	14	30	...	...	...	...	..	51	45	1.5	Died; carcinoma of tail of pancreas; metastasis to liver
...	22.3	+	..	..	...	...	...	...	34	36	30	7.0	Carcinoma of hepatic duct; extension to liver
330	43.2	+	12	..	95	137	130	42	38	33	22	...	Carcinoma of bile ducts; involvement of liver
142	26.5	+	10	15	86	105	99	19	28	26	26	11.9	After spontaneous decrease in jaundice
...	....	..	..	..	...	...	...	...	28	30	28	1.5	Adenocarcinoma of gallbladder; involvement of liver
25	....	..	11	50	117	140	145	28	..	30	25	5.0	Carcinoma of liver; marked jaundice
18	1.9	..	9	..	...	...	...	...	..	12	11	0.3	After spontaneous decrease in jaundice
210	30.4	+	12	..	90	121	129	34	24	24	20	10.8	Died; carcinoma of head of pancreas; metastasis to liver
...	....	..	6	11	...	...	...	...	15	20	13	1.5	Carcinoma of liver; biliary fistula with partial obstruction
					No	Pathologic	Diagnosis	Made					
...	28.2	+	..	..	86	140	...	54	..	44	34	...	Carcinoma of pancreas; diabetes; metastasis to liver
...	20.2	+	7	30	...	...	...	...	..	40	36	8.5	Malignant rectal polyp; metastasis to rectal shelf and liver
84	10.0	+	..	..	...	...	...	...	27	34	34	1.8	Inoperable carcinoma with ascites; metastasis to rectal shelf
100	15.6	+	16	30	103	117	115	14	30	32	22	0.9	Carcinoma of pancreas; metastasis to liver
120	30.2	+	12	..	93	192	200	107	30	28	24	8.0	Carcinoma of pancreas; diabetes; metastasis to liver
100	21.5	+	..	..	176	184	180	8	..	25	20	2.4	Carcinoma of pancreas; diabetes; metastasis to liver
...	....	..	7	..	...	...	...	...	23	20	15	6.0	Carcinoma of liver

Liver Without Jaundice

Bile Pigments (Serum)			Fructose Tolerance Blood Sugar, Mg. per Cent.				Phenoltetrachlorphthalein Dye in Serum, per Cent.				
Bile Index	Serum Bilirubin, Mg. per Cent.	Van den Bergh Direct Reaction	Normal	1 Hour	2 Hours	Change	15 Minutes	1 Hour	2 Hours	Dye in Urine, Mg.	Remarks
			Diagnosis	Confirmed	Pathologically						
12	2.8	0	...	...	...	..	18	17	14	0.0	Carcinoma of rectum; metastasis to liver
12	1.6	±	100	127	110	27	18	17	11	0.0	Carcinoma of gallbladder; metastasis to liver
..	0.9	0	...	...	...	..	12	6	3	0.0	Multiple tumors of liver, possibly sarcoma
..	5.2	+	...	...	...	..	12	..	..	...	Carcinoma of stomach; peritoneal carcinomatosis; metastasis to liver
4	1.1	0	...	...	...	..	..	7	2	0.0	Recurring carcinoma of rectum; metastasis to liver
..	0.2	0	...	...	...	..	8	6	2	0.2	Carcinoma of stomach; metastasis to liver
..	0.2	0	...	...	...	..	6	4	2	0.0	Carcinoma of stomach; invasion of liver
..	0.5	0	...	...	...	..	6	3	—	0.0	Carcinoma of colon; metastasis to liver
..	0.1	0	...	...	...	..	6	2	—	0.0	Carcinoma of rectum; metastasis to liver
6	1.0	0	...	...	...	..	5	2	—	0.0	Carcinoma of colon; metastasis to liver
..	0.1	0	...	...	...	..	5	2	—	0.0	Carcinoma of stomach; metastasis to liver
			No	Pathologic	Diagnosis	Made					
7	1.2	0	...	...	...	..	..	28	28	0.3	Carcinoma of liver
13	2.4	0	90	87	99	12	19	22	18	0.7	Carcinoma of stomach; probable metastasis to liver
16	2.6	0	...	...	...	..	12	22	22	1.3	
..	1.4	0	90	137	125	38	12	12	8	0.3	Carcinoma of liver
..	0.1	0	...	...	...	..	..	11	8	...	Carcinoma of colon; probable metastasis to liver
..	1.1	0	...	...	...	..	..	7	3	...	Adenocarcinoma of ovary; abdominal carcinomatosis; probable metastasis to liver
..	1.1	0	...	...	...	..	7	5	3	0.2	Carcinoma of liver
..	0.2	0	...	...	...	..	6	3	1	0.0	Carcinoma of hepatic flexure; probable involvement of liver

decreased to 16.2 mg. and the jaundice decreased to a corresponding degree. The toxemia, however, increased and during the last week the blood urea became elevated, reaching a level of over 200 mg. the day before death, September 9. During the last three days of life, a mass became palpable below the liver edge and was believed to be the gallbladder, but operation was not considered. At necropsy carcinoma of the head of the pancreas with obstruction of the common bile duct, metastasis to the liver and biliary cirrhosis were found.

This case presented many angles from the standpoint of diagnosis which was definitely made only at necropsy. The history is given in detail to emphasize the extreme difficulty of differential diagnosis in many of these cases. Catarrhal jaundice was first considered but did not account for the duration and intensity of the jaundice. The appearance of chills and fever with a moderate leukocytosis suggested the presence of cholangeitis. The possibility of cirrhosis was emphasized by the history of alcoholism. There was a questionable history of syphilis, but the negative Wassermann reaction and the lack of response to antisyphilitic treatment justified its exclusion. The extreme prostration and toxemia in the later stages of the disease resembled acute yellow atrophy. Carcinoma was considered, but in view of the patient's age and the absence of a demonstrable primary growth or of metastatic lesions it was thought to be unlikely. The final clinical diagnosis was obstructive jaundice with probable hepatitis, cirrhosis and cholangeitis. In this instance the laboratory findings were of no aid in differential diagnosis, for the high serum bilirubin and marked dye retention were not to be differentiated from those seen in obstructive jaundice due to stone or stricture of the common duct.

GROUP 2. METASTATIC CARCINOMA OF THE LIVER WITHOUT JAUNDICE

Rolleston [6] reports that in a series of cases of secondary malignant disease of the liver, the primary carcinoma in over 75 per cent. was situated in the stomach, colon, esophagus, pancreas or gallbladder. In the absence of obstruction to the bile duct, jaundice is uncommon and the clinical diagnosis of hepatic involvement by the malignant process is dependent largely on rapid and progressive enlargement, and particularly nodular enlargement, of the liver. Table 2 shows the changes in a series of eighteen cases of this type. In this group the laboratory tests are of the greatest clinical value.

CASE 15 (Table 2).—*Carcinoma of the rectum with metastatic nodules in the liver.*

A man, aged 73, was admitted to the Mayo Clinic, April 28, 1924, because of diarrhea and rectal pain of one year's duration. Slight bleeding had been observed for three or four months.

6. Rolleston, H. D.: Diseases of the Liver, Gallbladder and Bile Ducts, Ed. 2, London, Macmillan Company, 1924.

His weight on admission was 153 pounds (69.4 kg.), representing a loss of 17 pounds (7.7 kg.) in about four months. There was no jaundice, and no abdominal masses or tenderness were present. The liver was not palpable. A mass could be felt in the rectum, and on proctoscopic examination a large, fixed, partially obstructive lesion of the upper rectum and lower sigmoid was found. The serum bilirubin was 2.8. There was moderate retention of phenoltetrachlorphthalein with a reading of 17 per cent.

At operation a large carcinoma of the rectum was found. No definite local glandular involvement could be demonstrated, but there was extensive involvement of the liver with a large metastatic nodule on the superior anterior surface of the right lobe. On account of the obstruction a permanent colostomy was made.

In this case the constitutional symptoms were those of carcinoma, but there were no local signs to suggest invasion of the liver. The laboratory findings of a moderate dye retention and slight bilirubinemia suggested the extensive metastatic involvement of the liver revealed by operation.

CASE 16 (Table 2).—*Carcinoma of the gallbladder with invasion of the liver.*

A woman, aged 46, came to the Mayo Clinic, June 4, 1924, because of recurring attacks of pain under the right costal margin, accompanied by fever of eight months' duration. She had had typhoid fever in 1915. In November, 1923, she had an attack of severe pain in the right upper quadrant radiating to the lower abdomen. She was in bed for two or three days, during which time an egg-shaped mass was observed below the costal margin. The mass was continuously present after January, 1924, and the liver edge likewise became palpable. In May the liver was definitely enlarged; daily fever up to 102 degrees was observed, and the patient became much weaker and complained of a feeling of epigastric weight and discomfort after eating.

On admission the patient was very weak and emaciated. She weighed 106 pounds (48.1 kg.), having lost 20 pounds (9 kg.) during the last two months. There was no jaundice. The liver edge was felt 8 cm. below the costal margin, while a rounded mass extended 6 cm. below the liver margin. No glandular enlargement or palpable evidence of metastasis was found. The urine was normal except for the presence of a trace of bile. A test of the stool for bile was negative. The serum bilirubin was 1.6 mg. There was moderate retention of phenoltetrachlorphthalein, as the reading was 17 per cent. The patient was under observation ten days, during which time the temperature was irregularly elevated to a maximum of 102 degrees.

At operation, July 2, gallstones, carcinoma of the gallbladder and multiple metastatic nodules in the liver were found. It seemed likely that the fever was produced by infection in the gallbladder, but the extensive invasion of the liver and the weakened condition of the patient precluded further operative intervention. The pathologic diagnosis of a specimen removed from the liver was adenocarcinoma.

In this case the symptoms were preponderantly those of cholelithiasis with added infection. The laboratory tests, however, showed the presence of definite dye retention.

CASE 17 (Table 2).—*Multiple tumors of the liver.*

A woman, aged 42, was admitted to the Mayo Clinic, Dec. 6, 1924, because of a progressively increasing epigastric mass which she had noticed two months before. It was not painful although she had a certain sense of epigastric weight and discomfort. She had lost 15 pounds (6.8 kg.) because of voluntary dietary restriction although there was no definite food intolerance.

She was sallow but not icteric in appearance. The liver was greatly enlarged. There was a firm projecting mass in the right epigastrium, which appeared to be from 8 to 10 cm. in diameter. Smaller nodules were felt over the remainder of the surface of the liver, and there was a tumor in the pelvis 8 cm. in diameter. There was no enlargement of the superficial lymph nodes and a roentgenogram of the chest did not show metastatic invasion. The urine

contained albumin 3, but no bile. The serum bilirubin was 0.9 mg. There was moderate retention of phenoltetrachlorphthalein with a reading of 8 per cent.

An exploratory laparotomy was performed, December 17. There was one large, rounded solid tumor in the right lobe of the liver and several smaller tumors. It did not seem wise to remove a portion of the tumor for histologic examination, but the gross appearance was that of a sarcoma rather than that of a carcinoma. No primary source of the growth could be found in the abdomen. The pelvic mass was a dermoid of the left ovary.

The short history and the large nodular liver led to the clinical diagnosis of probable tumor of the liver. The moderate degree of dye retention furnished additional evidence in support of this diagnosis, which was confirmed by the findings at operation.

CASE 21 (Table 2).—*Carcinoma of the stomach with direct extension to the liver.*

A man, aged 48, was admitted, Dec. 8, 1924, because of stomach trouble of eighteen months' duration. Obstructive symptoms associated with progressive loss of 40 pounds (18.1 kg.) in weight, weakness and anemia developed.

The patient was anemic and cachectic in appearance. There was no jaundice, and the superficial lymph nodes were not enlarged. The liver was not palpable but there was a firm, fixed, nodular mass, apparently confluent with the stomach, in the left epigastrium. Gastric analysis revealed marked retention with a fasting content of 1,020 c.c. The total acidity of this fluid was 60 and of the free acids 0. Roentgenograms showed an obstructing lesion in the stomach. The serum bilirubin was 0.2. There was no retention of phenoltetrachlorphthalein; the reading was 4 per cent.

At operation a carcinoma of the stomach was found with extensive involvement of the adjoining lymph nodes. The carcinoma had perforated against the liver, extending locally into its substance. Resection of the entire growth was not possible.

There were no direct clinical evidences of hepatic involvement in this case, and the laboratory tests were negative. The carcinoma had invaded the liver by direct extension rather than by the blood or lymph stream. It is probable that the invasion was strictly localized and much less extensive than usual; this might explain the negative laboratory findings.

CASE 24 (Table 2).—*Carcinoma of the descending colon with metastatic nodules in the liver.*

A man, aged 36, was admitted to the Mayo Clinic, Oct. 20, 1924, because of constipation and left sided abdominal pain of ten months' duration. Blood clots had been passed by bowel on two or three occasions.

The patient was cachectic in appearance. His weight was 117 pounds (53.1 kg.), representing a loss of 43 pounds (19.5 kg.) in ten months. There was an enlarged gland in the right supraclavicular fossa. The liver edge was just palpable at the costal margin. Roentgenograms of the colon revealed a filling defect in the upper portion of the descending colon near the splenic flexure. The serum bilirubin was 1 mg. There was no retention of phenoltetrachlorphthalein; the reading was 2 per cent.

The supraclavicular gland was removed for examination and found to be inflammatory. At exploratory laparotomy a carcinoma of the descending colon was found; the glands along the spine were involved, and large and small carcinomatous nodules were present in the liver. Because of the extensive metastasis removal of the primary growth was not possible.

The patient's constitutional symptoms were those of neoplastic disease, but there were no direct clinical signs to indicate metastasis, and the laboratory findings were negative. There was neither bilirubinemia nor dye retention.

CASE 26 (Table 2).—*Carcinoma of the liver of unknown origin.*

A man, aged 70, was admitted to the Mayo Clinic, Nov. 1, 1924, because of dull epigastric pain of three months' duration. The pain usually came on one hour after eating, was confined to the epigastric and umbilical regions, and was not severe. Food or a bowel movement gave temporary relief. He had lost about 25 pounds (11.3 kg.) during the preceding three months. There was no jaundice.

There was no enlargement of the superficial lymph nodes. The liver was not palpable. The urine contained a trace of albumin. Roentgenograms of the stomach, colon and intestines failed to reveal any source of malignant disease. While the patient was under observation in the hospital, a mass became palpable in the epigastrium. It rapidly increased in size until it could readily be identified as the liver. November 21, there was marked retention of phenoltetrachlorphthalein with a reading of 28 per cent. The liver now extended to the umbilicus, and appeared to be nodular. Surgical intervention was not indicated.

The clinical diagnosis was carcinoma of the liver, although the primary focus was not identified. The laboratory studies demonstrated marked dye retention, a finding in complete accord with the clinical diagnosis.

GROUP 3. INTRA-ABDOMINAL CARCINOMA WITHOUT DEMONSTRABLE INVOLVEMENT OF THE LIVER

As a control of the cases reported in Tables 1 and 2, a series of seventeen cases of carcinoma of the stomach or colon without demonstrable involvement of the liver is presented (Table 3). In these cases the surgeon found the liver normal at the time of operation, which does not entirely exclude the presence of carcinomatous nodules since they may be either completely buried within the substance of the liver or, if external, so situated as not to be apparent at the time of operation. In either case, the degree of hepatic involvement would presumably be small. Three of the cases show a departure from the normal by the laboratory tests.

CASE 33 (Table 3).—*Carcinoma of the ascending colon.*

A man, aged 41, was admitted to the Mayo Clinic, June 6, 1924, because of attacks of colicky epigastric pain of nine months' duration. A month before admission he began to suffer from diarrhea, and clotted blood was observed in the stools. He had lost 30 pounds (13.6 kg.), and anemia became progressively more marked during the course of his illness.

He was weak and anemic in appearance. There was no jaundice and the liver was not palpable, but there was a poorly defined tender mass in the cecal area extending upward toward the costal margin. The superficial glands were not enlarged. Roentgenograms of the colon showed a filling defect in the ascending colon. The serum bilirubin was 1.9 mg. There was moderate retention of phenoltetrachlorphthalein with a reading of 10 per cent.

At operation a carcinoma of the posterior wall of the ascending colon which involved the cecal edge of the ileocecal valve was removed. There was local glandular involvement, but there were no visible evidences of invasion of the liver. The growth was removed; the patient recovered without complication, and his present condition is satisfactory.

CASE 34 (Table 3).—*Malignant rectal polyp.*

A physician, aged 47, was admitted to the Mayo Clinic, Nov. 15, 1924, because of blood in the stools. In 1909 varicose veins had been removed under chloroform anesthesia; following the operation he was jaundiced for several days but recovered completely. Blood had been noted in the stool six months before admission.

TABLE 3.—*Carcinoma*

Case	Date	Age, Years	Sex	Urine		Blood Count			Phenolsulphonephthalein, per Cent.	Blood Nitrogen Partition, Mg., per Cent.				
				Specific Gravity	Albumin	Hemoglobin, per Cent.	Erythrocytes, Millions	Leukocytes		Nonprotein Nitrogen	Urea	Uric Acid	Creatinin	Amino-Acid Nitrogen
33	6/16/24	41	M	1.023	0	40	2.72	7,100	50	55	76	2.9	1.4	61
34	11/19/24	47	M	1.027	0	75	4.47	8,100	..	37	22	2.8	1.7	62
35	4/11/24	67	F	1.027	1	70	4.03	5,200	..	25	21	2.4	1.4	63
36	9/11/24	56	M	1.019	1	34	3.01	5,600	..	28	24	3.3	1.8	58
37	10/17/24	50	M	1.016	0	40	3.22	7,400	30	28	29	3.5	1.8	82
38	4/ 3/24	58	M	1.022	1	50	3.16	6,500	60	35	45	3.3	1.6	57
39	11/ 7/23	50	M	1.020	1	46	3.40	9,000	..	..	36	2.8	2.9	...
40	4/25/24	48	M	1.011	0	71	4.09	5,800	..	37	50	....	1.7	69
41	24	43	M	1.030	1	67	3.97	5,200	..	38	23	4.4	1.7	64
42	11/ 2/23	47	M	1.019	0	45	3.33	10,500	..	..	38	3.1	2.4	...
43	5/22/24	64	M	1.025	2	62	3.96	11,200	55	33	29	1.9	1.8	54
44	11/ 8/23	67	M	1.022	0	50	3.50	5,000	55	..	21	....	....	...
45	6/ 2/24	60	M	1.027	1	72	4.28	5,100	..	29	39	2.1	1.8	62
46	8/ 2/24	76	M	1.027	0	75	4.32	8,000	..	38	31	2.2	1.8	...
47	9/ 3/24	55	M	1.035	0	73	4.25	8,000	..	32	38	2.0	1.8	55
48	10/24/23	59	M	1.022	0	..	....	.....	..	..	65	....	....	...
49	10/15/23	48	F	1.022	1	75	4.51	10,300	..	..	23	4.8	2.2	...

The patient was obese, weighing 243 pounds (110.2 kg.). There was no jaundice. The edge of the liver was palpable at the costal margin and was sharp. There were no superficial glandular enlargements. Proctoscopic examination revealed a large rectal polyp. The serum bilirubin was 2.2 mg. The dye reading of 7 per cent. indicated a slight retention of phenoltetrachlorphthalein.

At operation a carcinomatous polyp of the rectum was removed through a Kraske incision. There was no local glandular involvement. Direct examination of the liver was not possible.

In this case there was slight dye retention and bilirubinemia suggestive of hepatic disturbance. The rectal polyp was malignant, but there was no local evidence of extension of the growth. It is likely that the patient's jaundice in 1909, following chloroform anesthesia, had initiated changes which were responsible for the functional disturbances, although the patient had been symptomless since then. On the other hand, invasion of the liver cannot be ruled out.

CASE 42 (Table 3).—*Carcinoma of the stomach; secondary anemia.*

A man, aged 47, was admitted to the Mayo Clinic, Nov. 2, 1923. In February he had begun to have sharp abdominal pains shortly after eating. The liver and spleen were not palpable. The gastric acidity was normal, but old blood was present in the specimen. Roentgenograms disclosed carcinoma of the stomach high on the posterior wall. The phenoltetrachlorphthalein reading was 4 per cent. at the end of an hour. At exploration extensive local glandular involvement was found and the carcinoma was not removed. There was no evidence of involvement of the liver.

CASE 48 (Table 3).—*Carcinoma of the stomach.*

A man, aged 59, was admitted to the Mayo Clinic, Oct. 24, 1923. Six months before he had begun to have dull abdominal pain from one to two hours after eating, and he had lost weight and strength progressively. Retention vomiting had begun about a week before admission.

Without Liver Involvement

Bile Pigments (Serum)			Fructose Tolerance Blood Sugar, Mg. per Cent.				Phenol-tetrachlor-phthalein Dye in Serum, per Cent.			Dye in Urine, Mg.	Remarks
Bile Index	Serum Bilirubin, Mg. per Cent.	Van den Bergh Direct Reaction	Normal	1 Hour	2 Hours	Change	15 Minutes	1 Hour	2 Hours		
6	1.4	—	101	125	124	24	11	10	5	1.1	Carcinoma of ascending colon; local glandular involvement
..	...	..	...	...	...	..	11	7	5	0.8	Carcinomatous polyp of rectum
12	1.9	—	90	122	93	32	11	6	3	0.3	Carcinoma of stomach, inoperable; local glandular involvement
7	0.6	—	...	...	...	..	10	5	2	0.5	Carcinoma of cecum
8	0.5	—	...	...	...	..	10	4	1	0.6	Colloid carcinoma of stomach; peritoneal metastasis
9	0.1	—	87	122	135	48	8	4	1	0.6	Carcinoma of stomach, operable; no glandular involvement
..	...	..	...	...	...	..	7	4	2	0.4	Carcinomatous tumor of epigastrium
16	...	..	105	124	92	19	7	4	1	...	Carcinomatous ulcer of stomach; no glandular involvement
5	0.3	—	80	81	92	12	7	3	1	0.4	Carcinoma of stomach; marked glandular involvement
..	...	..	...	...	...	..	7	3	—	0.4	Carcinoma of stomach, inoperable
6	1.0	—	87	106	94	19	7	2	—	0.2	Carcinoma of stomach; slight local glandular involvement
...	...	..	...	...	...	..	6	2	—	...	Tumor of stomach
11	2.0	—	87	108	107	21	5	3	—	0.5	Carcinomatous ulcer of stomach; no glandular involvement
6	0.6	—	...	...	...	..	..	3	—	...	Carcinoma of pylorus; no glandular involvement
13	0.6	—	107	87	...	20	5	2	—	0.0	Carcinoma of rectosigmoid
..	...	..	...	...	...	..	4	—	—	0.0	Carcinomatous gastric ulcer
..	...	..	...	...	...	..	4	—	—	0.0	Carcinoma of stomach

On examination the patient was emaciated; he had lost 40 pounds (18.1 kg.) and was weak. The liver could not be palpated but there was a movable mass in the right upper abdominal quadrant. A test meal revealed a twelve-hour gastric retention of 1,150 c.c., and carcinoma at the pylorus was evident in the roentgenograms. The phenoltetrachlorphthalein reading was 2 per cent.

At operation a freely movable carcinomatous gastric ulcer involving the pyloric end of the stomach was found. There was no evidence of metastasis. A partial gastrectomy of the posterior Polya type was performed. The postoperative recovery was excellent.

INDIVIDUAL TESTS

Fructose Tolerance.—It has been reported that a lowered tolerance for carbohydrates accompanies many cases of carcinoma. Friedenwald and Grove[7] have recently reviewed the literature on this subject and found an apparently constant lowering of the glucose tolerance in a small series of cases of gastro-intestinal carcinoma. Kelly,[8] on the other hand, did not find significant changes in the glucose tolerance in his series of cases. The blood sugar in normal persons is less elevated following the ingestion of fructose than it is after the ingestion of an equal amount of glucose. A reduction in the fructose tolerance has

7. Friedenwald, J., and Grove, G. H.: The Blood Sugar Tolerance Test as an Aid in the Diagnosis of Gastro-Intestinal Cancer, Tr. A. Am. Phys. **35**:97, 1920.

8. Kelly, T. C.: Blood Sugar Retention in Carcinoma, Am. J. M. Sc. **169**:216 (Feb.) 1925.

been assumed, but not proved, to be a specific index to disturbances in the glycogenic activity of the liver.

In our series the fructose tolerance was definitely diminished in three cases and suggestively lowered in three, in a total of eight tests on patients with carcinoma involving the liver and associated with jaundice. Clinical diabetes also was present in two of the cases, showing a reduced carbohydrate tolerance. Tests were made on three patients with carcinoma involving the liver without jaundice; the fructose tolerance was reduced in one. In those patients with carcinoma without proved metastasis to the liver there were two with a lowered fructose tolerance in a total of eight. Because of the irregularity with which positive tests were secured and the frequent association of pancreatic disease and clinical diabetes with the disturbance of the liver in this series of cases, we question the clinical value of the fructose tolerance test as a specific clinical index to the functional efficiency of the liver.

Blood Sugar Level.—Friedenwald and Grove report an increase in the fasting blood sugar level in the cases of carcinoma studied by them. They also quote previous workers who made similar observations. In our series of cases, there was no significant elevation of the fasting blood sugar above the normal. A high reading, 176 mg. per cent., was obtained in only one instance, and in that case there were clinical evidences of diabetes secondary to a carcinoma of the head of the pancreas.

Nitrogen Partition of the Blood.—There were no changes in the nonprotein nitrogen of the blood that could be attributed directly to the carcinomatous process. In five cases the blood urea was greater than 50 mg. In one case (Case 18, Table 2), there was an associated pyelonephritis and a terminal increase in the blood urea from 70 to 466 mg. In another (Case 6, Table 1), an increase in the blood urea from 20 to 200 mg. was associated with the development of a fatal toxemia, and was not affected by the intravenous administration of glucose. Three of the patients with carcinoma had a blood urea level of 50 mg. or more on admission. In all, however, this was associated with partial intestinal or pyloric obstruction with consequent dehydration, and the blood urea promptly returned to normal following the administration of an adequate amount of sodium chlorid solution. The general level of the blood urea was perhaps slightly lower in those cases with jaundice, 25.5 mg., than in Groups 2 and 3, from 29.3 to 36.4 mg., although all were within the lower limits of normal. The changes, however, were not sufficient to be of clinical significance in any individual case.

Noteworthy changes were not observed in the other nonprotein nitrogenous constituents of the blood studied. The total nonprotein nitrogen, uric acid, creatinin aminonitrogen all came within the limits of normal.

Bile Pigments.—The cases were arbitrarily grouped according to the presence or absence of clinical jaundice. When icterus was manifest the serum bilirubin was uniformly increased, the highest reading being 71.6 mg. (Table 1). In all these cases the icterus index was increased and the direct van den Bergh reaction was obtained, the findings being entirely comparable to those in the series of cases of obstructive jaundice previously reported.

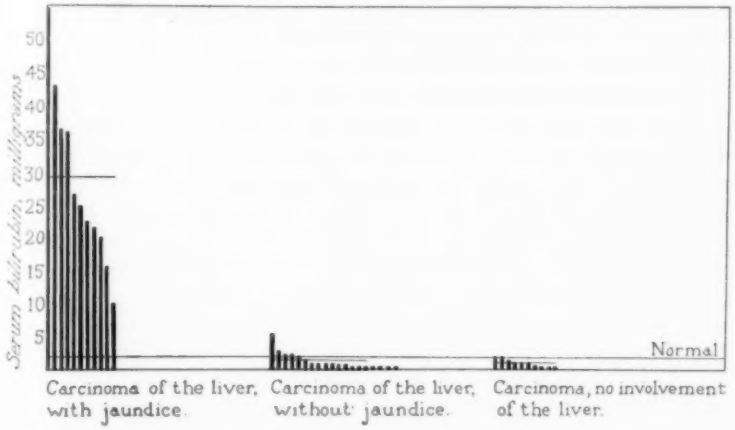

Fig. 1.—Variation in the serum bilirubin in the different types of carcinoma studied. The maximal normal level is indicated, and in each group the average value is shown by the horizontal line.

Normal values for the serum bilirubin were obtained in the third group of carcinoma cases, those without evident metastatic involvement of the liver. Latent icterus was demonstrable in three of the eighteen cases of carcinoma with metastatic involvement of the liver reported in Table 2. In one the serum bilirubin was 5.6 mg. and the direct van den Bergh reaction was obtained. The skin and sclerotics of this patient had a subicteric tint. In the other two patients, clinical evidences of jaundice were wanting. The serum bilirubin readings were normal in the other cases of this group.

Phenoltetrachlorphthalein.—We have previously shown the consistent occurrence of a marked retention of phenoltetrachlorphthalein both in patients with obstructive jaundice and in animals following ligation of the common bile duct. In such cases the bile retention

apparently conditions the dye retention. The results of the test in patients with carcinomatous involvement of the liver and jaundice were similar to those in obstructive jaundice of any origin. Retention of dye in the blood stream was marked. In this series of cases, the maximal reading in the one-hour sample was 51 per cent. and all the cases showed a reading of 20 per cent. or over. This is in marked contrast to the maximal normal reading of 3 per cent.

The second group of cases is more striking even than the first. In a large proportion of these cases (eleven out of eighteen) with metastasis to the liver, there was definite retention of phenoltetrachlorphthalein even in the absence of bile retention, as shown by the values for the serum bilirubin (Table 2). The degree of dye retention was unquestioned, although less striking than that observed in the jaundiced cases. The maximal reading in the one-hour specimen was 28 per cent. A questionable degree of dye retention was observed in two. However, a normal response was obtained in five of the cases.

The determination of the minimal degree of dye retention that is of clinical significance is important in evaluating the phenoltetrachlorphthalein test in a study such as this. Rosenthal gives the normal readings for phenoltetrachlorphthalein as 7 per cent. at fifteen minutes, 3 per cent. at one hour, and none at two hours. These represent maximal figures in normal persons. There is considerable variation in hospital cases, and numerous cases are seen in which the readings are slightly higher without there being definite clinical evidence of hepatic disease. We believe that readings of at least 10 per cent. at fifteen minutes, 5 per cent. at one hour, and 3 per cent. at two hours, are required before they can be considered significant of hepatic disturbance. Lesser degrees of dye retention are of questionable significance.

Of the seventeen cases of carcinoma without demonstrable metastasis to the liver, three showed slight dye retention and two others showed questionable retention; in the other twelve elimination curves were normal. In none of these cases did the urine specimen contain more than 1 mg. of phenoltetrachlorphthalein without there being at the same time definite retention of dye in the blood stream.

COMMENT

In order properly to evaluate the clinical usefulness of laboratory tests in the demonstration of carcinomatous invasion of the liver, a clear understanding is necessary both of the physiologic activity of the liver and of the pathologic processes concerned in the spread of a malignant tumor. Carcinoma, in general, spreads along lymphatic channels, first involving the adjacent lymph nodes. These may be so situated as to permit of complete surgical removal, although this is often impossible. The liver may be affected by direct extension, as from carcinoma of

the gallbladder or stomach, or through lymphatic or vascular channels. The secondary lesions may be single or multiple, and may vary in size from microscopic aggregations of growing tumor cells to huge masses that replace or destroy the normal structure of the organ. In the one instance the most careful pathologic examination may be necessary to find evidence of the invasion of the liver, while in another the clinical diagnosis may be unmistakable.

Many observers have emphasized the large functional reserve or margin of safety of the liver. Experimentally, we have found that when chloroform is injected intraportally, according to the method of Schultz, Hall and Baker,[9] it produces multiple areas of necrosis in the liver without affecting the remainder of the organ. In such animals, tests may show normal function. On the other hand, prolonged chloroform anesthesia or the intramuscular injection of chloroform produces uniform parenchymal changes throughout the liver. Marked functional disturbances may readily be demonstrated in these animals.

Similar factors may undoubtedly determine the effect of malignant invasion of the liver. Apart from obstruction to the biliary passages and the production of jaundice, the degree of damage produced by the malignant process will depend, more or less quantitatively, on the extent of the destruction of hepatic tissue. Until there is some infringement of the normal reserve, functional disturbance cannot be expected.

Robertson [10] has emphasized the possibility that tumors in the liver, perhaps through interference with the vascular supply, may produce degenerative changes in the parenchymal tissue over an area quite disproportionate to the size of the metastatic nodule. It is apparent, therefore, that while a normal response to a laboratory test will not wholly exclude the possibility of malignant disease, a positive test may be of great value in indicating the presence of hepatic involvement.

In the first group of cases (carcinoma with manifest jaundice), the functional changes were characteristic and definite. These changes are identical with those observed in the cases of obstructive jaundice previously reported. No distinction is possible on the basis of the laboratory tests between cases of obstructive jaundice due to carcinoma of the head of the pancreas and the present cases of carcinomatous involvement of the liver with jaundice. The differential diagnosis may be extremely difficult in cases of complete biliary obstruction. As in Cases 3 and 5 (Table 1) fluctuations in the degree of jaundice from time to time and the intermittent appearance of bile in the stools do not wholly exclude the possibility of carcinoma, although they are more

9. Schultz, E. W.; Hall, E. M., and Baker, H. V.: Repair of the Liver Following the Injection of Chloroform into the Portal System, J. M. Res. **44**:207-230 (Dec.) 1923.

10. Robertson, H. E.: Personal communication to the author.

frequently associated with obstruction due to stone. The effects of the bile retention dominate the laboratory findings, and while the evidence of hepatic disturbance is definite, the tests do not distinguish between the different etiologic agents possibly concerned. The laboratory tests are of value, however, in furnishing quantitative evidence of the extent of the changes produced by the jaundice. The serum bilirubin is of particular value in that it furnishes a much more sensitive and accurate index to the degree of bile retention than does any clinical impression regarding the color of the skin or conjunctivae. Particularly when the obstruction to the biliary passages is of neoplastic origin may the clinical evidences of malignancy be lacking. In the absence of such evidence,

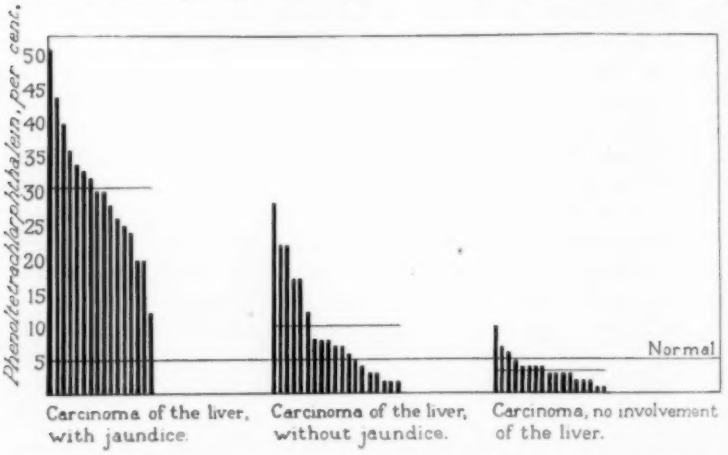

Fig. 2.—Variations in the degree of phenoltetrachlorphthalein retention in the different types of carcinoma studied. The maximal value level is indicated, and in each group the average value is shown by the horizontal line.

the exact diagnosis is obtained only at operation or necropsy. It is in just such cases that some reliable test indicating the presence of carcinoma somewhere within the body would be of inestimable value.

In the absence of jaundice, the clinical diagnosis of invasion of the liver by a malignant process is largely dependent on the demonstration of enlargement of the liver or of nodules over its surface. These signs are often lacking and the clinical diagnosis is impossible. In the majority of the cases of this type, as shown in Table 2, the phenoltetrachlorphthalein test shows moderate retention of the dye, which we believe to be especially significant. In the presence of clinical evidence of hepatic involvement it is pathognomonic. If the liver is not palpable and carcinoma can be demonstrated elsewhere, the possibility of hepatic involvement must be seriously considered. In a few of these cases,

an increase in the serum bilirubin or latent jaundice was demonstrated. This finding affords valuable confirmatory evidence of the hepatic involvement shown by the phenoltetrachlorphthalein test. As we have pointed out, a negative test does not exclude hepatic damage, but in several of the cases the phenoltetrachlorphthalein test furnished the only evidence of metastasis to the liver. It is in such cases that functional tests assume their greatest significance, since they affect clinical judgment in regard to diagnosis, prognosis and treatment.

Normal responses to the different tests were obtained in practically all of the cases of carcinoma without apparent involvement of the liver. One patient with a history of jaundice following chloroform anesthesia showed slight dye retention; this serves to emphasize the necessity of excluding other forms of hepatic injury before concluding that positive tests truly indicate malignant disease of the liver. Similarly, toxic influences may explain the results in the other three cases in which there was slight delay in the removal of phenoltetrachlorphthalein from the blood stream. It is also possible that in these cases the dye retention was evidence of metastatic involvement of the liver that was not visible to the surgeon. If so, this would further emphasize the value of the phenoltetrachlorphthalein test.

CONCLUSIONS

The determination of the serum bilirubin and the phenoltetrachlorphthalein test promise to be of very definite assistance in the study of patients with abdominal carcinoma and suspected malignant disease of the liver, although the greater number of the other tests for hepatic function studied fail to show significant changes or sufficiently specific changes to be of any great clinical value.

When extension of the malignant process to the liver is accompanied by jaundice, as from obstruction of the extrahepatic bile passages, it is direct evidence of a disturbance in the normal physiologic activity of the liver. In such cases functional tests and the determination of the serum bilirubin are of value in that they furnish a sensitive and quantitative means of studying the effects of the jaundice. The tests are of less value in differential diagnosis, for the jaundice dominates both the clinical picture and the laboratory findings. The jaundice associated with malignant disease in the liver is not to be differentiated by these tests from obstructive jaundice from any cause.

It is in the study of patients with carcinoma involving the liver without jaundice that functional tests are of greatest value, particularly the phenoltetrachlorphthalein test. In the cases in which there was clinical evidence of malignant disease of the liver, confirmatory results were obtained by functional studies. In the absence of clinical evidence of hepatic involvement, the phenoltetrachlorphthalein test may furnish

the only evidence of the existence of metastatic nodules in the liver. Positive tests are not obtained in all cases of metastatic involvement of the liver, for positive tests are a measure of the interference with the activity of the liver as a whole, and the latter is an organ with a large margin of safety. For this reason, however, positive tests when obtained become doubly significant.

There are definite limitations to the use of functional tests in the study of hepatic disturbances, and in our experience the extreme difficulty attending the early clinical recognition of malignant involvement of the liver and biliary passage was again manifest. We believe that the serum bilirubin and the phenoltetrachlorphthalein tests are of clinical value in the early diagnosis of carcinoma of the liver and biliary tract.

NUTRITIONAL CHANGES IN EXOPHTHALMIC GOITER

THE EFFECT OF LUGOL'S SOLUTION *

CYRUS C. STURGIS, M.D.
AND
JAMES A. GREENE, M.D.
BOSTON

The body weight is undoubtedly one of our most valuable indexes of nutrition and indicates clearly, when determined over a long period, the balance between the energy supplied to the body in the form of food and the total energy expended as heat and muscular movements. As exophthalmic goiter is one of the few conditions in which the basal metabolism is characteristically elevated, the relation between the food intake, energy expenditure and body weight in this disease should be of considerable interest. It is well known that every normal individual expends a definite amount of energy depending on sex, age, height, weight, food intake and muscular activity. To provide for this an equal number of calories in the form of food must be ingested or the individual will consume his own tissues and a loss in body weight will follow. If a quantity of food in excess of the energy requirement is eaten, the surplus calories will be stored in the body chiefly in the form of fat and carbohydrate, and there will therefore be a gain in body weight. In a normal person, a diet that is inadequate in calories first results in a loss of body weight. Following this there is a drop in the basal metabolism or energy requirement. In other words, a normal person, within certain limits, can adjust his coloric production to meet his caloric intake in the form of food. For example, Benedict [1] reports a group of normal young men who consumed from 3,200 to 3,600 calories daily while leading an active life. Their diet was reduced until the body weight loss averaged 12 per cent. At this level their basal energy requirement had diminished 18 per cent. and these men could then maintain their body weight on an average of 1,950 calories daily. Joslin [2] has calculated that, in normal persons, for each 1 per cent. loss in weight there is a percentage decrease in the metabolism of 1.8. The fall in the basal metabolism with the undernutrition may be regarded as an attempt to prevent further loss of weight by lowering the total energy expenditure.

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* From the medical clinic of the Peter Bent Brigham Hospital.

1. Benedict, F. G.: Metabolism During Starvation and Undernutrition, New York M. J. **115**:249 (March) 1922.

2. Joslin, E. P.: Diabetic Metabolism with High and Low Diets, Carnegie Institution of Washington Publication No. 323, 1923, p. 72.

The basal energy requirement, that is, the amount of energy expended by a person at complete rest in bed is easily calculated from the Aub and DuBois [3] standards. For example, a man, aged 35, height 176 cm. and weight 75 kg., requires approximately 1,800 calories. To this should be added 10 per cent., or 180 calories, for the specific dynamic action of food. If the person is up and about and pursuing an occupation that involves a moderate amount of muscular exertion, an addition of 50 per cent. of his basal caloric requirement should be added. The total number of calories required by such a person per twenty-four hours is, therefore, 2,880. This coincides roughly with numerous observations on the amount of food eaten by normal persons under these conditions.

The foregoing considerations are preliminary to a discussion of the metabolism or energy requirement in exophthalmic goiter and its relation to the food consumption and body weight in this condition. If we consider the example previously cited of a man, aged 35, height 176 cm. and weight 75 kg., in whom the normal basal requirement is 1,800 calories per twenty-four hours, and in addition assume the basal metabolism to be 50 per cent. above normal, which is a fair estimate in a moderately severe instance of exophthalmic goiter, it is possible to calculate the dietary requirements of such a patient. Following an extended study [4] of the food consumption and basal metabolism in a patient with exophthalmic goiter, in this hospital in 1922, it was concluded that a caloric intake averaging 84 per cent. greater than the basal metabolism was necessary in order to maintain weight. Boothby and Sandiford [5] have made extensive studies of the total metabolism in nine patients with exophthalmic goiter and give in a detailed table, among other information, the basal metabolism and food intake per twenty-four hours for each patient before surgical measures were instituted. These subjects were at complete rest in bed or up from two to three hours daily. The average intake was 3,517 calories per twenty-four hours, or 78 calories per kilogram of body weight. Each patient remained in a positive nitrogen balance and there was a very slight gain in weight, averaging 75 gm. per twenty-four hours for the group. In order to accomplish this, it was necessary for each patient to consume an average of 3,517 calories daily, which was an intake 90

3. Aub, J. C., and DuBois, E. F.: Clinical Calorimetry, XIX, The Basal Metabolism of Old Men, Arch. Int. Med. **19**:823 (May) 1917.

4. Sturgis, C. C.: Observations on One Hundred and Ninety-Two Consecutive Days of the Basal Metabolism, Food Intake, Pulse Rate and Body Weight in a Patient With Exophthalmic Goiter, Arch. Int. Med. **32**:50 (July) 1923.

5. Boothby, W. M., and Sandiford, Irene: The Total and Nitrogenous Metabolism in Exophthalmic Goiter, J. A. M. A. **81**:795 (Sept. 8) 1923.

per cent. greater than the average basal metabolism of the group. For the maintenance of body weight it seems reasonable to conclude that, in addition to the extra energy required by the increase in the basal metabolism, it is necessary to add another increment of from 75 to 100 per cent. of the basal metabolism on account of the specific dynamic action of the food eaten and the excessive activity of these patients even though they are resting in bed most of the time. If the energy requirements are calculated under such conditions, we should add 900 calories to the normal basal requirement of 1,800 calories of the person previously considered for the increase of 50 per cent. in the basal metabolism, and 85 per cent. of the elevated basal, or 2,295 calories, as an average for the muscular movements of a patient in bed and the specific dynamic action of food. The total amount, therefore, is found to equal 4,995 calories. Such an estimate indicates the number of calories necessary to furnish a patient of this age, height and weight, having a basal metabolism 50 per cent. above normal, with sufficient food in order to maintain body weight while resting in bed. It would be difficult to estimate, with any degree of accuracy, the dietary requirement necessary if such a person were carrying on an occupation requiring moderate or heavy exertion. Boothby and Plummer [6] have reported that in hyperthyroidism a given amount of work requires almost twice the energy expenditure that it does in normal persons, or, in other words, such a patient is very inefficient in the performance of mechanical work, which is a matter of considerable importance in estimating their total energy expenditure. A conservative estimate of the caloric requirements under these circumstances would be in the vicinity of 6,000 calories or more, an amount that represents a very large quantity of food and requires an unusually good appetite before it can be ingested.

The various factors governing the caloric requirement in normal persons who are undernourished are not effectual in patients with exophthalmic goiter. While undernutrition in an otherwise normal person is followed by a fall in the basal metabolism, the same is not true in a patient with exophthalmic goiter, for in this condition, although many pounds of body weight may be lost, the constant stimulus to cellular metabolism characteristic of the disease elevates the basal metabolism to a level that may reach 75 or 100 per cent. above normal. Therefore, although the food intake may be diminished, the basal metabolism or energy expenditure remains at a level often greatly above normal, and as a result the patient's own body tissues are burned to such an extent that a striking loss of body weight results unless a very large quantity of food is eaten.

6. Plummer, H. S., and Boothby, W. M.: The Cost of Work in Exophthalmic Goiter, Am. J. Physiol. **63**:406, 1922-1923.

DuBois [7] has emphasized that the normal subject's appetite unconsciously balances the bodily needs and maintains a practically constant weight in many people with extraordinary exactness. If a large percentage of normal persons meet their energy requirements by consuming an approximately exact quantity of food, the question may be asked how adequately are the increased energy requirements met in a patient with exophthalmic goiter and an increased metabolism.

In order to secure information on this question, the histories of sixty-five patients have been considered in order to ascertain the amount of weight lost and to gain an approximate idea of the food intake from their own statements concerning their appetites over the duration of the disease. Sufficient data was obtainable on which an opinion might be based in fifty-two of the sixty-five histories. This information revealed that in 86.5 per cent., or 45 patients, of the 52, there was a loss of body weight averaging 11.9 kg. (26.3 pounds) in an average length of time of 11.9 months. Six patients stated that there had been no variation in body weight, and in one it was claimed that the weight had increased 11 pounds (5 kg.). When we consider the rather striking weight loss in such a high percentage of these patients over a relatively short interval, it is interesting to obtain an estimate of their food intake. The patient's appetite was classified as ravenous, increased, normal or decreased. In eighteen, or 34.6 per cent., the appetite was considered as ravenous; in eleven, or 21.2 per cent., as increased, in seventeen, or 32.7 per cent., as normal, and in six, or 11.5 per cent., as decreased. In summary it can be said that in a total of forty-six patients, or 88.5 per cent., the appetite was regarded as ravenous, increased or normal. When it is considered that 86.5 per cent. of the same fifty-two patients lost an average of 11.9 kg. (26.3 pounds) in 11.9 months, it emphasizes in a very striking manner the inability of most patients with exophthalmic goiter, when leading a more or less normal life, to consume a diet adequate in calories to provide for the greatly augmented energy expenditure. In a small number of patients with exophthalmic goiter having a basal metabolism elevated above normal limits, however, it is apparently possible to ingest sufficient food to maintain body weight, and even in rare instances to increase it, despite the large energy requirements. Under the latter circumstances the appetite must be extraordinarily large. If the patient's food consumption is persistently small over a long interval and yet his activities are not limited, the loss of weight is often remarkable, resembling the decrease frequently observed in severe untreated diabetes. In the last analysis, variation in body weight in patients with exophthalmic goiter is dependent on the balance

7. DuBois, Eugene: Basal Metabolism in Health and Disease, Ed. 1, Philadelphia, Lea and Febiger, 1924, p. 181.

between the amount of energy which is available to the body in the form of food and the total energy expended, which depends chiefly on the amount of muscular exertion and the level of the basal metabolism. From a consideration of the available data it is apparent that most patients, although they have a large appetite, cannot supply their increased energy requirements and therefore lose weight, while a very small percentage can ingest sufficient food to maintain and even, in rare instances, to gain weight.

The rather unusual history of a weight loss and a good or excessive appetite is not frequently encountered in clinical medicine and is information of a particularly definite nature which suggests the possibility of an increased metabolism; it is therefore of value from a diagnostic standpoint. Under ordinary circumstances undernutrition, as it is usually observed in clinical medicine, is associated with a poor appetite and a diminished food intake. The striking exceptions to this are found in diabetes and exophthalmic goiter. In these conditions similar degrees of undernutrition are frequently observed. Joslin [8] found, in a study of 200 patients with diabetes, that the average weight loss was 12 kg. (26.4 pounds), which represented the difference between the maximum weight before the onset of the disease and the weight when the patient was first observed. In the group of patients with exophthalmic goiter who lost weight (86.5 per cent. of the group), the decrease was almost precisely the same, as the average loss was 11.9 kg. (26.3 pounds). Joslin [9] reports that the respiratory quotients of the severe cases of diabetes studied at the Nutrition Laboratory between 1908 and 1911 averaged 0.73, which indicated that very small amounts of carbohydrate were being utilized. It is interesting to note that the respiratory quotients of this group of patients with exophthalmic goiter averaged approximately the same, the figure being 0.75. Although in individual instances no great importance can be attached to small changes in the respiratory quotient, they are of considerable significance when the quotients of a large group of patients are considered. Quotients of 0.73 and 0.75 in these two groups of patients indicate that 8.4 per cent. and 15.6 per cent., respectively, of the total calories are derived from carbohydrate, whereas the average quotient of 0.83, which was found in 236 normal persons in the Harris and Benedict [10] series, means that 43.8 per cent. of the calories produced were due to the combustion of carbohydrate. In severe, uncomplicated diabetes a low respiratory quotient

8. Joslin, E. P.: The Prevention of Diabetes Mellitus, J. A. M. A. **76**:79 (Jan. 8) 1921.

9. Joslin, E. P. (Footnote 2, p. 165).

10. Harris and Benedict: Carnegie Institution of Washington Publication No. 279, 1919, pp. 40 and 44, Tables C and D, quoted by Joslin (Footnote 2, p. 167).

is expected as in a subject with this disease there is an inability to utilize carbohydrate. In exophthalmic goiter there is no impairment of the ability to burn carbohydrate but owing to the increased energy requirements of the disease, the glycogen stores are constantly called on and are therefore greatly depleted, which is further evidence of undernutrition in this condition.

COMPARISON OF BODY WEIGHTS OF PATIENTS WITH EXOPHTHALMIC GOITER WITH STANDARD WEIGHT TABLE BASED ON HEIGHT, AGE AND SEX

To secure further information concerning the nutrition of patients with exophthalmic goiter, the body weights of sixty-five such patients were compared with the Medico-Actuarial Weight Tables,[11] which have been utilized by Joslin [8] in a study of the body weight of patients with diabetes. It was impossible to use the more recent weight standards proposed by Dreyer and Hanson [12] which are based on stem length and chest girth, as these measurements were not available in this group of patients. The comparison of the actual body weights of the sixty-five patients with the weight tables showed that twelve, or 18 per cent., averaged 6.8 per cent. greater than is considered normal according to the standards used. One patient of this group appeared obese and was actually 15.9 kg. (35 pounds) over her estimated normal. One patient of the 65, or 1.5 per cent., had a body weight that coincided exactly with the calculated normal. In fifty-two of the sixty-five patients, or 80 per cent., the body weight was found to average 18.2 per cent. below the normal. Judging from clinical experience and from these observations it seems safe to conclude that the body weight of about 20 per cent. of all patients with exophthalmic goiter will be normal or slightly above and that they will appear well nourished; in rare instances, they may even be slightly obese. The remaining 80 per cent. will give evidences of loss of body weight, which will average, if a group is considered collectively, about 18 per cent. of their body weight before the onset of the disease.

In making these conclusions, the question quite properly arises of the reliability of the normal weight tables which were used. It is, of course, impossible to estimate precisely the ideal weight of a person basing the calculations on height, age and sex. The normal standards that were utilized are considered among the most reliable available. An

11. Medico-Actuarial Mortality Investigation, Association of Life Ins. Med. Directors and Actuarial Soc. of America, New York **1**:37 (Table 3), 66 (Table 8), 1921.

12. Dreyer, Georges; and Hanson, G. F.: The Assessment of Physical Fitness, New York, Paul B. Hoeber, 1921.

individual variation of 10 per cent. below normal cannot be considered significant, but if a group of sufficient size is considered collectively and their average is found to be 10 per cent. below the normal, it indicates a definite abnormal variation.[13] In order to ascertain the accuracy of these normal standards we have compared the actual body weights of a group of normal persons, male and female, with the normal weights given by the tables. In thirty-five males between the ages of 19 and 54 years, the actual body weight averaged 2.9 per cent. below the weights given in the standard tables. In thirty-five females, between the ages of 20 and 53 years, the variations between the actual were 3 per cent. below the calculated body weight. The average variation from the normal standard of the entire group of seventy people was 2.97 per cent. below the calculated body weight. These deviations from the normal standard seem surprisingly small and appear to justify the conclusions concerning the body weight of patients with hyperthyroidism which have been given above. Additional data is available furthermore which indicates the accuracy of these standards when applied to groups of persons. Our conclusion has been that 80 per cent. of the group of patients with exophthalmic goiter have a body weight almost 20 per cent. below the normal standard when they present themselves at this hospital for examination. The question may be asked, How many normal persons will be 20 per cent. below these standards? This may be answered by a consideration of the facts shown by the New England Mutual Life Insurance Company Mortality Investigation between the years 1844 and 1905, when it was found that of the 136,240 insured healthy persons only 4.6 per cent. were 20 per cent. underweight [8] according to these standards. These comparisons therefore indicate that considerable significance can be attached to the statistics concerning body weight in patients with exophthalmic goiter when a group of sufficient size is considered.

It is apparent, then, that a large majority (80 per cent.) of these patients, when they appear at this hospital for treatment after having had exophthalmic goiter for an average interval of 16.1 months, show rather striking undernutrition. This results from an inability to provide by their diet for the high caloric requirement due to a characteristically high basal metabolic rate, an inefficiency in the performance of mechanical movements, and a loss of the normal adjustment mechanism which makes possible a drop in the basal metabolism as undernutrition develops. When the dietary requirements are inadequately met, the patient's reserve stores of fat and carbohydrate are drawn on as sources of energy. Ultimately if these fail to provide sufficient energy, body pro-

13. Root, H. F., and Miles, W. R.: Physical Measurements of Diabetic Patients, J. Metabolic Res. **2**:190 (Aug.) 1922.

tein is consumed as food, and under these circumstances a loss of nitrogen results. According to our figures such a patient may be represented theoretically by a person whose weight has decreased from a normal of 68.2 kg. (150 pounds) to 58 kg. (123 pounds), a loss of 18.2 per cent., throughout an illness of 16.1 months. During this interval it is reasonable to suppose that periods of nitrogen loss may be present owing to an inadequate diet. Such a patient's condition, with reference to undernutrition, is somewhat comparable to that of a subject who has undergone a complete fast for twenty-nine days and as a result suffers a loss in body weight of 20.1 per cent., which is the average loss in that length of time as indicated by Benedict [14] in his resumé of fasting. This amount is similar to the 18.2 per cent. loss which occurs in many patients with exophthalmic goiter, although in the latter the decrease in weight is distributed over a much longer period. The foregoing summary emphasizes the magnitude of the loss in body weight which frequently occurs in patients with this disease. More important still, it is such an undernourished patient who comes to a hospital and in a variable period of time, often too brief, undergoes a major operation on the thyroid gland.

LOSS OF BODY WEIGHT IN PATIENTS WITH EXOPHTHALMIC GOITER FOLLOWING MAJOR OPERATIONS ON THE THYROID GLAND

The importance of an additional loss of weight following operative procedures in an already undernourished patient led to a careful survey of the percentage loss of weight in these patients following partial or subtotal thyroidectomy. The question of determining the change in body weight and analyzing accurately the factors responsible for this change is not as simple as it may appear on first inspection. In studying these patients the preoperative body weight, without clothes, was recorded at the time of the last determination of the basal metabolism, which was usually within two or three days before operation. The patient was weighed, usually between ten and eleven days after the operation, when the first metabolism following the operation was determined. This weight loss recorded ten or eleven days after the operation probably does not indicate accurately the maximum as by this time the postoperative febrile reaction had disappeared; food could be taken in fair amounts, and the metabolism was usually normal, according to the observations of Segall and Means[15] It seems a reasonable estimate to

14. Benedict, F. G.: A Study of Prolonged Fasting, Carnegie Institute of Washington Publication No. 203, 1915, p. 80.

15. Segall, H. N., and Means, J. H.: The Immediate Effect of Subtotal Thyroidectomy in Toxic Goiter; Daily Basal Metabolism and Pulse Observations, Arch. Surg. 8:176 (Jan.) 1924.

assume that the maximum loss of body weight occurs from the fourth to the sixth postoperative day, and that several pounds may be gained between that time and the day on which the patient is actually weighed. The error resulting from weighing the patient on the tenth or eleventh postoperative day would therefore tend to minimize the loss of weight following operation.

TABLE 1.—*Percentage Variation in Body Weight Following Subtotal Thyroidectomy in Thirty-Six Patients with Exophthalmic Goiter Who Were Not Treated with Lugol's Solution Prior to Operation*

Case	Variation in Body Weight Following Operation, per Cent.*	Postoperative Day Weight Was Determined	Metabolism Before Operation	Metabolism After Operation†
1	−6.4	18	+29	− 3
2	−5.3	11	+43	+18
3	−6.2	3	+33	+75
4	−2.9	11	+37	+16
5	−7.7	6	+41	+16
6	−9.7	8	+38	+11
7	−6.8	14	+29	+13
8	−3.5	11	+28	− 9
9	−6.9	6	+63	+43
10	−4.0	11	+21	+ 8
11	−5.3	17	‡	‡
12	−1.5	11	‡	‡
13	−2.4	18	‡	‡
14	−5.0	10	‡	‡
15	−3.1	12	‡	‡
16	−5.5	14	‡	‡
17	−3.8	7	+54	+30
18	−5.2	10	+33	+14
19	−6.4	11	+34	+12
20	−4.3	10	+26	+39
21	−4.2	13	+44	+40
22	−5.4	8	+37	+ 7
23	−6.7	10	+31	− 3
24	−3.5	10	+13	− 5
25	−5.6	7	+40	....
26	−5.9	16	+50	+24
27	−1.0	15	+29	+10
28	−7.9	12	+34	+24
29	−5.6	5	+17	− 4
30	−4.6	12	+44	+21
31	−11.9	15	+49	+28
32	−12.5	13	+71	+29
33	−2.9	10	+55	+33
34	−4.1	11	+39	+ 3
35	−6.0	15	+26	+12
36	±0.0	9	+53	+36
Average	−5.2	11	+39	+18

* The minus sign indicates a loss of weight.
† The metabolism determination was made on the same day the postoperative body weight was obtained, as indicated in the second column.
‡ The basal metabolism was not determined in these patients.

The loss of body weight following a major operation on the thyroid gland in thirty-six consecutive patients with exophthalmic goiter is shown in Table 1. It is to be emphasized that every single patient lost in body weight except one, in whom it was unchanged. The greatest loss was 12.5 per cent., while the smallest was 1 per cent. The average loss was 5.2 per cent. in an average of eleven days, which would represent, for example, a decrease of 3.5 kg. (7.7 pounds) in a patient whose original body weight was 68.2 kg. (150 pounds) immediately before the

operation. This weight loss assumes more importance when one considers that these patients are usually undernourished before the operation and suffer an additional weight loss of considerable extent in a few days following the operation. It is possible to illustrate the changes in nutrition more strikingly if the percentage changes are applied to actual body weights. For instance, if a patient weighed 68.2 kg. (150 pounds) before the onset of the disease and then lost 12.9 kg. (26.3 pounds), which was the average amount lost in 86.5 per cent. of these patients according to their own statements, the weight on admission to the hospital would be 56.1 kg. (123 pounds). Eleven days after operation, with an average loss of 5.2 per cent., or 2.9 kg. (6.4 pounds), the patient's weight would be 53.3 kg. (117.3 pounds). Therefore, the total loss of weight estimated to occur in a patient with exophthalmic goiter whose original weight before the onset of the disease was 68.2 kg. (150 pounds) and who fell into the group of undernourished patients, would be 14.9 kg. (32.7 pounds), or 21.8 per cent., including the decrease in weight following the operation.

ANALYSIS OF FACTORS RESPONSIBLE FOR LOSS OF WEIGHT IN PATIENTS WITH EXOPHTHALMIC GOITER FOLLOWING OPERATION

Among the important factors which must be considered and which may be responsible for fluctuations in body weight following operation may be mentioned: the level of the basal metabolism immediately before and after the operation; the extent of the postoperative febrile reaction; the ability of the patient to ingest food, which may be influenced by postoperative nausea and vomiting or difficulty in swallowing due to trauma about the neck; the type of anesthetic, postoperative acidosis, and changes in the water balance of the body. All of these factors may be of importance in the same patient, but in a consideration of this entire group an attempt has been made to determine the relative importance of each of these.

The most rapid fluctuations in body weight are associated with alteration in the water balance of the body. Striking illustrations of this are found in the data obtained by Professor William G. Anderson of the Yale University Gymnasium, who observed that a football player lost 6.4 kg. (14 pounds) in one hour and ten minutes, a Marathon runner lost 3.9 kg. (8.5 pounds) in three hours, and a member of a college eight-oared boat crew lost 2.5 kg. (5.5 pounds) in twenty-two minutes.[16] The decrease in body weight was due in each instance to increased destruction of body tissue and loss of water. The former was

16. Anderson, W. G., quoted by Benedict, F. G., and Joslin, E. P.: A Study of Metabolism in Severe Diabetes, Carnegie Institution of Washington Publication No. 176, 1912, p. 96.

insignificant as indicated by the calculations of Benedict and Joslin,[16] who estimated that during an hour of strenuous muscular exertion, similar to the exercise of a football game, not more than 100 gm. (about one-fourth pound) of dry organic material would undergo combustion in the body. Subtracting this from the total loss of weight, there is left 6.3 kg. (13.75 pounds) of lost weight due to water. It is of importance to note that in these persons there was no fluid intake or a very slight one during the intervals of exercise, and that the body weight of the football player returned to normal inside of forty-eight hours following food and drink. While there may have been fever and excessive sweating for a few days during the postoperative febrile period in the patients observed, there is no indication that an excessive quantity of fluid was lost from the body. Immediately after the operation the fluid intake was maintained in the vicinity of 2,500 c.c. every twenty-four hours by means of subpectoral saline injections and dextrose solution by rectum. In addition, sips of water by mouth were permitted, beginning a few hours after the operation. The only factor of significance concerning the water balance is a possible accumulation of edema due to cardiac failure, which may be intensified as a result of the operation. A few of these patients had some evidence of cardiac failure with moderate edema before the operation, but they were not operated on until the edema had disappeared as a result of rest in bed and digitalis. While there was no visible edema after operation, it is well known that approximately 10 pounds (4.5 kg.) fluid may collect in the body tissue without giving definite evidence in the form of "pitting." The collection of edema may introduce an error in the weight change, as it may tend to mask any real loss of weight. In this group of patients, however, there was no indication that a change in the body weight due to the accumulation or loss of edema was of importance.

Previous to the collection of data, it was thought that the effect of the various anesthetics might be related to the magnitude of the weight loss following operation. Apparently the type of anesthetic had no significance in this respect, as shown by the following data, although the number of patients was too small to afford conclusive evidence. Of the thirty-six patients, eighteen were given ether and the average weight loss was 4.9 per cent.; eight patients were operated on under procain and the average weight loss was 4.64 per cent.; eight were given nitrous oxid and oxygen and had an average weight loss of 6.51 per cent.; one had a combination anesthesia of nitrous oxid, oxygen and procain which was followed by a loss in weight of 6.4 per cent.; and one had nitrous oxid, oxygen and ether, with a loss in weight of 5.29 per cent. It seems logical to assume that the administration of ether as an anesthetic will result in the most severe postoperative symptoms, which will

be followed by the greatest loss in body weight. This is not supported by the foregoing data, nor is there any conclusive evidence for or against any one particular anesthetic as far as the loss of body weight is concerned.

Goetsch and Browder [17] have emphasized that acidosis is very prone to occur after operations on the thyroid gland and that acetone may appear in small amounts in the urine even immediately before operation. According to these investigators, the postoperative acidosis is responsible for marked restlessness, often distressing nausea and vomiting, headache, irritability of the bowel with diarrhea, a dry tongue, parched lips, acetone odor to the breath and often large amounts of acetone in the urine. Mild postoperative acidosis, as evidenced by acetone bodies in the urine, has been observed in a fair number of these patients, although never in any instance has a severe acidosis been encountered. It appears difficult to ascertain to what extent the postoperative symptoms are due to the formation of ketone bodies, but it does not seem possible that they can be a factor of the greatest importance in the loss of weight following thyroid operations. The development of an acidosis does emphasize the undernutrition of these patients and suggests very strongly that the glycogen reserves of the body in patients with exophthalmic goiter are usually very low. Therefore, for several days after the operation, when very little or no food is being consumed, the available body glycogen is completely exhausted and an acidosis may result.

The inability to take food owing to nausea, vomiting and difficulty in swallowing as a result of trauma about the neck is undoubtedly of importance and accounts, in part at least, for the loss of some weight following major thyroid operations. It is not the sole factor, as indicated by a study of the postoperative loss of weight in a series of twenty-five patients with colloid goiter and nontoxic adenoma, all of whom had either a normal metabolism or no clinical evidence that their basal metabolism was increased. Twenty-two of these patients were given ether as an anesthetic. Reference to Table 2 shows that the average loss in body weight in these patients averaged only 2.47 per cent. as compared to an average loss of 5.2 per cent. in patients with exophthalmic goiter and an elevated metabolism. As almost all of the patients with colloid goiters and nontoxic adenomas had ether and the operative procedures were at least comparable in extent to that employed in exophthalmic goiter, it seems logical to assume that the postoperative nausea, vomiting and difficulty in taking food following the operation would be similar in the two conditions. So, while postoperative complications are

17. Goetsch, E., and Browder, E. J.: Studies on Thyroid Disorders, IV, Intravenous Administration of Glucose Solution in Treatment of Acidosis Following Thyroid Operations, New York State J. Med. **22**:469 (Oct.) 1922.

of importance in exophthalmic goiter, they explain only approximately one half of the weight loss and it becomes necessary to attribute the remainder to some other factor.

It has been previously emphasized that patients with exophthalmic goiter who lose body weight do so because their energy expenditure is at such a high level that their food intake fails to supply an adequate number of calories and the patient consumes his own body tissues. Following subtotal or partial thyroidectomy the food intake is practically nil for a period of several days and it is usually eight or ten days or longer before the diet is taken in normal amount. In the series of thirty-six patients shown in Table 1 the metabolism was elevated in each

TABLE 2.—*Percentage Loss of Weight in Twenty-Five Patients with Colloid Goiter and Nontoxic Adenomas*

Case	Variation in Body Weight Following Operation,* per Cent.	Postoperative Day Weight Was Determined
1	—2.51	14
2	—1.66	3
3	—0.41	8
4	—2.64	10
5	—2.72	15
6	—3.88	7
7	—3.34	9
8	—3.54	8
9	—1.17	10
10	—2.36	12
11	—3.64	7
12	—5.1	4
13	—2.76	8
14	—2.94	5
15	—6.00	8
16	—3.68	6
17	—2.56	8
18	—4.04	11
19	—2.24	8
20	—1.35	11
21	—4.57	7
22	—3.82	12
23	+3.41	8
24	+1.86	12
25	+2.87	12
Average	—2.47	8.9

* The minus sign indicates a loss, the plus sign a gain in body weight.

instance before the operation, the average being 39 per cent. above the normal. While the basal metabolism returned to normal within from two to three weeks in almost every instance following the operation, it did remain elevated in a number of patients for a period of ten days, and during the first three or four postoperative days it was probably elevated above the preoperative level, owing to the characteristic febrile reaction after these operations, at which time the body temperature may reach 101 degrees or higher. At this time the highly undesirable combination of a markedly elevated metabolism associated with no food intake, or a very low one, exists in a patient. This is obviously the most important cause of the weight loss following operation in these patients.

That a loss in body weight commensurate with that actually observed could result in a few days in a patient without food and with an elevated basal metabolism is easily verified by calculation. It has been previously estimated that a patient, of average size, having a basal metabolism of 50 per cent. above normal would require 4,995 calories to maintain body weight while resting in bed. For several days immediately after the operation it is reasonable to suppose that additional restlessness and the usual postoperative fever would easily raise the caloric requirement to even a higher level. During the first three postoperative days ordinarily a negligible amount of food is ingested, while the minimum energy expenditure for this interval is assumed to be 4,995 calories a day, or 14,985 calories for the total period. Joslin [18] has calculated that each kilogram of body weight lost represents 3,766 calories, having based his figures on the amount of weight lost and the total metabolism in a subject who underwent a total fast of thirty-one days at the Nutrition Laboratory in Boston. Assuming that it is correct to apply this figure to a patient with exophthalmic goiter, it is apparent that a total loss of 3.98 kg. (8.7 pounds) would result in three days under these conditions. If the subject's original weight before the operation was 68.2 kg. (150 pounds), this would represent a loss of almost 6 per cent., which is in approximate agreement with the average actual recorded loss of 5.2 per cent. in the thirty-six patients of Table 1.

Additional evidence that corroborates the suggestion that the high metabolism and small food intake are responsible for the great weight loss is to be found in Table 3, which shows the postoperative decrease in body weight in twenty-eight consecutive patients with exophthalmic goiter who had been treated with Lugol's solution for an interval of from ten to fifteen days prior to the operation. Following the administration of iodin the characteristic drop in metabolism, which resulted in an average basal metabolism of + 21 for the group just prior to the operation, was obtained.[19] This is in contrast to the group of thirty-six patients who were not treated with Lugol's solution and in whom the average metabolism was + 39 just before the operation. In the group of twenty-eight patients treated with Lugol's solution, five, or 17.8 per cent., actually weighed more on the tenth postoperative day than they had before the operation, while the average loss of weight for the entire group following operation was 2 per cent., as contrasted to a loss of 5.2 per cent. for

18. Joslin (Footnote 2, p. 68).

19. Boothby, W. M., and Plummer, H. S.: The Value of Iodin in Exophthalmic Goiter, J. Iowa M. Soc. **14**:66 (Feb.) 1923. Starr, Paul; Segall, H. N., and Means, J. H.: The Effect of Iodin in Exophthalmic Goiter, Arch. Int. Med. **34**:355 (Sept.) 1924. Mason, E. H.: The Use of Lugol's Solution in the Treatment of Exophthalmic Goiter, Canad. M. A. J. **14**:219 (March) 1924. King, B. T.: The Treatment of Exophthalmic Goiter With Iodin, Northwest Med. **23**:165 (April) 1924.

the group who were not treated with Lugol's solution and therefore entered the operation with an elevated metabolism. When applied to actual figures these percentage values show that if a patient's body weight before the operation is 68.2 kg. (150 pounds) and he is not treated with Lugol's solution, and therefore enters the operation with an elevated metabolism, the loss of body weight will average 5.2 per cent., or 3.5 kg. (7.8 pounds), which will result in a body weight of 64.6 kg. (142.2 pounds) on the tenth postoperative day. If the patient

TABLE 3.—*Percentage Variation in Body Weight Following Subtotal Thyroidectomy in Twenty-Eight Patients with Exophthalmic Goiter Who Had Been Treated with Lugol's Solution Prior to Operation* *

Case	Variation in Body Weight Following Operation, per Cent.†	Postoperative Day Weight Was Determined	Metabolism Before Operation	Metabolism After Operation‡
1	−1.5	12	+19	− 2
2	−2.6	10	+31	+12
3	−3.7	18	+23	+ 9
4	−3.1	8	+16	+10
5	−1.5	8	+ 5	+ 7
6	−2.0	8	+15	+ 4
7	−4.5	10	+25	+ 5
8	−4.8	9	+20	+22
9	−2.5	3	+50	+59
10	−0.8	11	+32	+10
11	−0.3	11	+25	+ 3
12	−0.8	17	+11	+29
13	−7.0	12	+15	+18
14	−3.1	7	+14	−10
15	−1.0	4	+28	± 0
16	−1.6	8	+16	− 5
17	−0.7	11	+14	−13
18	−3.8	9	+24	+11
19	−3.5	6	+26	+ 7
20	−3.9	18	+ 4	+17
21	−3.6	7	+10	+ 1
22	−6.3	14	+28	+16
23	−8.2	5	+25	+34
24	+8.7	11	+28	− 7
25	+0.4	10	+20	−11
26	+0.6	10	+15	−17
27	+2.5	9	+13	+13
28	+0.9	9	+35	+14
Average	−2.0	9.8	+21	+ 8.4

* The initial metabolism of this group averaged +55 on admission to the hospital and before Lugol's solution was given. This is approximately the same as the average initial metabolism of the group shown in Table 2.

† The minus sign indicates a loss of weight.

‡ The metabolism determination was made on the same day the postoperative body weight was obtained, as indicated in the second column.

is treated with Lugol's solution, the calculated weight loss will be 2 per cent. on the tenth postoperative day and the patient's body weight will be 66.8 kg. (147 pounds), which is a significant difference from 64.6 kg. (142.2 pounds), as indicated above.

The practical suggestions that may be deduced from these observations are obvious. If a patient falls into the undernourished group, as indicated by a comparison of the actual with the normal weight tables, and in addition gives a history of a fairly large loss of weight, treatment should first be directed toward the undernutrition, a phase of the dis-

ease that is frequently ignored. If the treatment is to be a subtotal thyroidectomy, then the patient should be under observation for a variable period, depending on the degree of undernutrition. If the body nutrition is good and there has been no loss of weight or only a small amount, then a period of rest in the hospital of only ten days or two weeks is usually sufficient. At this time Lugol's solution should be administered, and after the third or fourth day the basal metabolism should be determined daily in order to ascertain its lowest level, at which time the subtotal thyroidectomy should be performed. Previously it had been our custom to precede the major operation with one or more preliminary ligations of the thyroid arteries. As the preoperative administration of Lugol's solution has come into greater use, these ligations have been eliminated, with no untoward results. The preliminary period of rest in bed also permits careful study of the heart and the institution of proper therapy when indicated. A patient who is markedly undernourished presents a problem of considerable importance. Such a patient should be under observation resting in bed for a period of four or five weeks or longer. During this time every attempt should be made to give a diet averaging between 75 and 100 per cent. greater than the basal metabolism per twenty-four hours. At the same time an effort should be made to decrease the patient's caloric requirements by enforcing strict rest in bed. The management of the diet in patients with exophthalmic goiter requires the greatest skill and resourcefulness. The production of nausea by using foods too sweet or too high in fat content should be avoided. The only food which some foreign patients desire is often of a special character and is obtained with difficulty. Frequently the patient who will not eat can be made to eat by the simple procedure of carefully investigating the character of the articles of diet desired and supplying food that is relished. When possible, charting of the caloric intake is worth while, as it furnishes an accurate index of the food consumed and is an added incentive to the patients, the nurses and attending physicians to keep the caloric intake at a high level.

It is usually not desirable to begin Lugol's solution in these undernourished patients until the probable time of operation is known, unless the patient's condition is serious and demands immediate attention. Otherwise, the optimal effect will probably occur from the iodin therapy in ten or twelve days, at which time the gain in weight may not have been as much as desired. If a further interval is required in order to gain weight, the possibility that the metabolism might gradually rise, despite the administration of iodin, must be considered under these circumstances. The most rational procedure is to keep the patient at complete rest in bed for several weeks, forcing him to take as much food as possible. If the weight curve at that time is upward and the

patient had made a substantial gain, then Lugol's solution should be given for an average of about ten days, in order to reduce the metabolism to the lowest level in preparation for operation.

The postoperative treatment of such patients should be followed with the greatest care. Lugol's solution should, of course, be continued on the day of the operation, by rectum if necessary, and for several weeks following the operation. In addition every attempt should be made to supply an adequate fluid intake. This may be accomplished by giving sips of water or other liquids within a few hours after the operation, provided nausea and vomiting are not contraindications. If necessary 1,500 c.c. of physiologic sodium chlorid solution may be given subpectorally in twenty-four hours. It is also desirable to provide as much food as can be taken immediately following the operation. This may be accomplished by giving orange juice and other drinks containing carbohydrates at the earliest possible moment, due care being taken that nausea and vomiting are not induced. It is of decided advantage, furthermore, to give as many calories as possible by rectum, although it has been said that only from 25 to 35 per cent. of nourishment necessary to maintain nitrogenous equilibrium and weight can be given in this manner.[20] Rectal feeding is best accomplished by giving 1,000 c c. of water containing 50 gm. of dextrose and 50 gm. of ethyl alcohol in doses of 250 c.c. from every four to six hours. Although the caloric content of this solution is not great (550 calories per thousand cubic centimeters of solution) it is the optimal mixture from a caloric content that is non-irritating and can be retained and absorbed by the patient. Solid food and a general well balanced high caloric diet should be taken by the patient as soon as possible during convalescence. The combination of these simple measures, in an attempt to supply adequate fluid and as much nutriment as possible, and the reduction in the metabolism accomplished by Lugol's solution diminish the tendency of the patients to lose weight following operation. The postoperative loss of weight is of great importance, as the patients are already undernourished, and the measures to conserve weight at this time add safety to the operation and decrease the length of convalescence.

SUMMARY

The relation between the food intake, basal metabolism and body weight in patients with exophthalmic goiter has been emphasized. From our present observations, which are in accord with those made previously, it is concluded that in order to maintain body weight while resting in bed the food intake in a patient with exophthalmic goiter and

20. Carter, H. S.: Artificial Methods of Feeding in Endocrinology and Metabolism, New York, D. Appleton & Co., 1922, **3**:810.

an elevated metabolism must be at least from 75 to 100 per cent. greater than the basal metabolism. A study of the histories of sixty-five patients with exophthalmic goiter indicated that although 88.5 per cent. of them had an appetite which was classified as ravenous, increased or normal, yet 86.5 per cent. of these patients gave a history of a loss of 11.9 kg. (26.3 pounds) of body weight in 11.9 months. A comparison of the body weight of these sixty-five patients with the standard weight tables shows that 80.2 per cent. averaged 18.2 per cent. below normal when they first appeared at the hospital. A study of the alteration in body weight following operation in thirty-six patients who had not been treated with Lugol's solution and therefore were operated on with an elevated metabolism, averaging + 39, showed that all except one, in whom the weight was unchanged, lost an average of 5.2 per cent. of their preoperative body weight in an average of eleven days after the operation. The factors responsible for this loss of weight might be several, but it was concluded that the most important was the combination of the inability of the patient to consume normal quantities of food and the elevated metabolism, which may remain high for a period of ten days or longer following thyroidectomy. A second group of twenty-eight patients, who had been treated with Lugol's solution and in whom the metabolism was reduced to an average of + 21 before the operation, was studied. In these patients, 18 per cent. had actually gained weight by the tenth postoperative day, and the loss of weight for the entire group averaged only 2 per cent. This loss is even less than the average decrease in body weight following operation in a group of twenty-five patients with colloid goiters and nontoxic adenomas.

GLYCEMIA AS A GUIDE IN THE TREATMENT OF DIABETES MELLITUS

THE PRACTICABILITY OF ROUTINE EXAMINATIONS OF SMALL, EFFECTIVELY PRESERVED SPECIMENS OF BLOOD DRAWN BY THE PATIENT *

WALTER H. NADLER, M.D.; PAUL STARR, M.D.
AND
GERTRUDE TUKEY, B.S.
CHICAGO

The necessity of maintaining normal blood sugar levels in diabetes mellitus has been urged by a few writers who believe that hyperglycemia, in the treated as in the untreated aglycosuric patient, permits overwork of and resultant damage to the pancreas.[1] This goal in treatment conforms with the modern conception of diabetes and with our knowledge of the nature of insulin. The necessity of preventing hyperglycemia is not, however, admitted by all writers even though there is practical agreement as to the necessity of keeping patients aglycosuric. Some authors consider control of glycemia an unnecessary refinement. Many emphasize the importance of blood sugar estimations in diagnosis but consider frequent determinations as a guide in treatment inconvenient and impracticable because of the additional expense and discomfort to the patient. These objections have been largely overcome by the introduction of accurate micro methods that permit the use of small samples of capillary blood, easily obtained from the finger or ear, and the use of preservatives, which make unnecessary the immediate examination of specimens. That routine determinations with the micro method are practicable has already been demonstrated by Ordway.[2]

The inconvenience to the patient of repeated visits to the laboratory in a fasting state for the purpose of having blood samples drawn seemed to us the chief difficulty in carrying on frequent routine tests. In the case of patients who lived at a distance, such examinations were impossible. A method was therefore sought which would permit the patient himself to collect small samples of blood that could be effectively preserved and brought or mailed to the laboratory. The procedure to be described, adopted after a trial of various micro methods and of preservatives described in the literature, successfully meets these require-

* From the Department of Internal Medicine, Northwestern University Medical School, and Wesley Memorial Hospital.

1. Campbell, W. R., and MacLeod, J. J. R.: Insulin, Medicine **3**:262 (Aug.) 1924. McCaskey, G. W.: Renal Glucose Threshold and Its Bearing on Insulin Treatment of Diabetes, New York M. J. **118**:215-217 (Aug. 15) 1923.
2. Ordway: Tr. A. Am. Phys. **39**:313, 1924.

ments. It is an adaptation of methods already reported. The Folin-Wu method of blood sugar estimation using 0.1 c.c. of blood, described by Randles and Grigg,[3] seemed the most satisfactory of the micro tests and was employed with only slight alteration in technic. For preservation the mixture of sodium fluorid and thymol recommended by Sander [4] was found to be effective. It was used in a slightly different proportion in order to insure the presence of sufficient thymol in the smaller amount of mixture required for the micro method.

ACCURACY OF THE MICRO METHOD

Randles and Grigg reported that with more than 100 samples run by both the regular Folin-Wu method and the 0.1 c.c. modification the agreement in results was entirely satisfactory, from the clinical point of view at least. They found an average difference of about 5 per cent. The following slight alterations in technic were made by us: The 0.1 c.c. of blood (measured in a 0.1 c.c. pipet made by Emil Greiner for the Kramer-Gittleman micro method) was expelled into the bottom of the centrifuge tube containing the sulphuric acid solution and the pipet was washed out several times with the acid. After being stirred, and with the stirring rod still in place, the sodium tungstate solution was added by placing the tip of the pipet on the stirring rod near the surface of the acid.

Readings obtained in our laboratory with the micro method as compared with the regular Folin-Wu method appear in Table 1.

For values less than 100 mg. per hundred cubic centimeters of blood, the average difference was 0.0066 gm.; for values between 100 and 200 mg., the difference averaged 0.0056 gm.; and for values between 200 and 400 mg. per hundred cubic centimeters of blood, the average difference in glucose was 0.0077 gm. per hundred cubic centimeters of blood. The micro method seems, therefore, to be accurate for clinical purposes as stated by Randles and Grigg.

PRESERVATIVE AND ANTICOAGULANT

Randles and Grigg collected their blood samples in vials containing 2 drops of a 1 per cent. solution of potassium oxalate which was dried in the vial. As a preservative, effective for from two to three days, they used formaldehyd solution added by means of a toothpick dipped in the solution and stirred with the few drops of blood after the excess of formaldehyd had been shaken off.

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3. Randles, F. S., and Grigg, W. K.: Estimations of Blood Sugar by the Folin-Wu Method, Using 0.1 C.c. of Blood, J. A. M. A. **82**:684-686 (March 1) 1924.

4. Sander, F. V.: The Preservation of Blood for Chemical Analysis, J. Biol. Chem. **58**:1 (Nov.) 1923.

More permanent and satisfactory preservation was obtained by us with sodium fluorid and thymol. Sander recommended a mixture of ten parts of sodium fluorid and one part of thymol, the whole having been finely powdered and passed several times through a 100 mesh sieve. Using relatively large amounts of blood, he found that a mixture of 0.01 gm. of sodium fluorid and 0.001 gm. of thymol to each cubic centimeter of blood was a preservative for from six to fourteen days. For 0.2 c.c. of blood we, at first, used 5 mg. of the 10:1 mixture; more than that amount of sodium fluorid was found to interfere with the precipitation of the protein. It was then found with the 10:1 proportion that occasionally a blood was not preserved, probably because

TABLE 1.—*Accuracy of the Micro Method*

Regular Folin-Wu (2 C.c. of Blood)	Micro (0.1 C.c. of Blood)	Difference
0.069	0.062	−0.007
0.075	0.082	+0.007
0.075	0.085	+0.010
0.077	0.069	−0.008
0.079	0.072	−0.007
0.085	0.090	+0.005
0.087	0.074	−0.013
0.088	0.079	−0.009
0.090	0.093	+0.003
0.091	0.083	−0.008
0.097	0.100	+0.003
0.099	0.100	+0.001
0.101	0.102	+0.001
0.102	0.090	−0.012
0.104	0.102	−0.002
0.118	0.118	0.000
0.128	0.136	+0.008
0.128	0.123	−0.005
0.139	0.138	−0.001
0.149	0.154	+0.005
0.161	0.180	+0.019
0.185	0.182	−0.003
0.215	0.220	+0.005
0.222	0.232	+0.010
0.253	0.256	+0.003
0.266	0.270	+0.004
0.323	0.328	+0.005
0.339	0.345	+0.006
0.350	0.334	−0.016
0.357	0.370	+0.013

the mixture, which is a mechanical one, was not complete enough to insure the presence of sufficient thymol in the small amount (5 mg.) of the mixture used. The proportion was therefore changed to 10:2, with the result that all of the bloods were perfectly preserved. We recommend this proportion for the use of small amounts of blood.

Table 2 shows the effect of incubation on micro samples taken from the veins of two normal persons. After seven and eight days' incubation, respectively, the final difference was very slight and well within the limits of error of the micro method.

In addition to the preservative series kept in the incubator, micro samples of blood from a diabetic patient were mailed to various parts of

the country after the sugar content was determined. Table 3 shows the variations found in the samples on their return to the laboratory from six to eleven days after mailing. The differences were well within the limits of error of the method.

COLLECTION AND PRESERVATION

Small, wide mouthed vials (7 by 12 mm.) fitted tightly with rubber stoppers were used for collecting the samples of blood. To these vials had been added from a small glass spoon the mixture of sodium fluorid and thymol. Patients were taught to prick their own finger tips with an automatic spring lancet, the point of which had been sharpened and shaped to resemble a Hagedorn needle. The points were set at 3 mm.

TABLE 2.—*Preservation of Bloods Incubated at 37 Degrees Centigrade*

Micro Samples (0.2 C.c.) of Same Blood Preserved with 5 Mg. of Sodium Fluorid and 0.5 Mg. of Thymol			
Before Incubation	Third Day	Seventh Day	Final Difference
0.100	0.091	0.102	+0.002
0.100	0.096	0.098	−0.002
0.104	0.080	0.102	−0.002
0.098	0.098	0.104	+0.006

Micro Samples (0.2 C.c.) of Another Blood Preserved with 4 Mg. of Sodium Fluorid and About 0.5 Mg. of Thymol Added Separately			
Before Incubation	Fifth Day	Eighth Day	Final Difference
0.0865	0.092	0.080	+0.0015
0.091	0.093	0.095	+0.004

TABLE 3.—*Preservation of Bloods Sent Through the Mails**

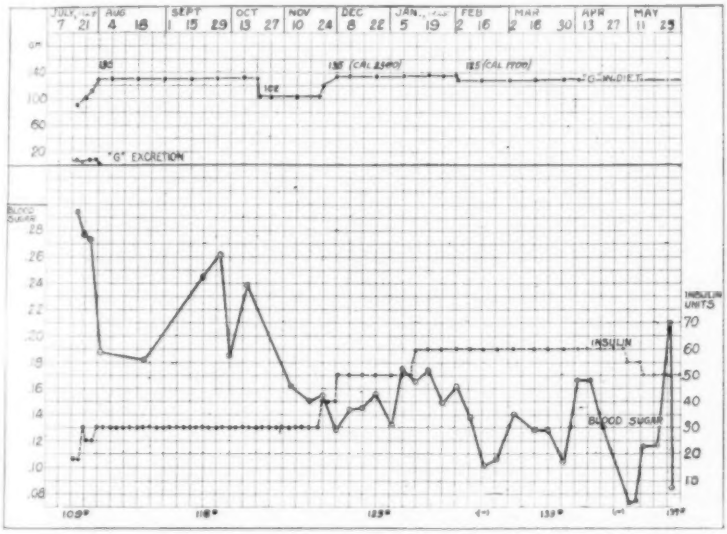
Destination	Days to and from	Blood Sugar (Gm. per 100 C.c.)
Control (not mailed)	0	0.198
	0	0.206
Hudson, N. Y.	6	0.192
	6	0.198
Birmingham, Ala.	7	0.200
	7	0.204
Los Angeles	9	0.192
	9	0.195
Prineville, Ore.	11	0.192
	11	0.197

* Ten micro portions (0.2 c.c.) of the same blood were preserved with about 5 mg. of a 5 : 1 mixture of sodium fluorid and thymol. Duplicate samples were mailed and tested on their return.

in order that the resulting cut would insure a free flow of blood. By gentle massage of the finger, about 8 drops could usually be obtained. After the collection of about 0.2 c.c., when the vial was half full, the blood was stirred thoroughly with a tooth pick and the vial stoppered. It was then inclosed in a small mailing tube and sent to the laboratory. For routine observations bloods were taken by the patients at home before breakfast (and insulin). It was found that even the most nervous patients willingly and easily learned to collect these samples and that the procedure had a valuable disciplinary effect.

COMMENT

The practicability of regular, routine estimation of sugar in small samples of blood drawn by patients at home has been demonstrated in a series of diabetic cases during a period of from six to eleven months. The accompanying chart illustrates the clinical course from the standpoint of the fasting blood sugar of one of the patients treated with insulin. Tests were made at weekly intervals for the most part, but more frequent readings were made in patients with marked hyperglycemia, especially when associated with tuberculosis and other complications. Analyses of the preserved bloods were usually deferred until



Blood sugar curve of patient, aged 31, who was first seen in May, 1922, seven weeks after the onset of symptoms. For over a year he remained aglycosuric on a high protein, low fat and carbohydrate diet. He then increased his diet to the point of permitting occasional glycosuria. Later, after a "cold," glycosuria became constant. In July, 1924, insulin treatment was started and observation of fasting blood sugars was begun. Blood samples were drawn at home by the patient and sent to the laboratory. The thirty units of insulin daily freed the urine of glucose but 60 units were required to maintain a normal blood sugar level. Recently 50 units have sufficed. Temporary elevations have occurred without apparent cause. The weight of the patient has increased from 100 to 137 pounds (45.4 to 62.1 kg.).

eight or ten specimens had accumulated. As a result of these determinations, a more accurate check on the efficacy of dietary and insulin control has been obtained and variations in metabolism have been more quickly discovered than would have been possible from examination of

the urine alone. The accuracy of the readings is not affected by the utilization of finger instead of venous blood since it has been shown that, in the fasting state, the sugar content of capillary blood, which is virtually arterial, is practically the same as that of venous blood.[5] The procedure described is likewise applicable when more frequent estimations are desired as in carbohydrate and glucose tolerance tests and in all day curves. In the interpretation of such curves, however, consideration must be taken of the fact that the glucose in finger (arterial) blood may be appreciably higher than that of corresponding venous blood.[6]

SUMMARY

Patients can be taught to collect from their fingers and bring or mail to the laboratory a few drops (about 0.2 c.c.) of blood which is effectively preserved and kept from clotting by a mixture of sodium fluorid and thymol recommended by Sander (5 mg. of sodium fluorid and 1 mg. of thymol are used). Estimations of sugar, accurate for clinical purposes, can be made at a convenient time with the micro modification of the Folin-Wu method described by Randles and Grigg. The procedure described is practicable. It provides a method of routine observation of the fasting blood sugar level of diabetic patients.

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5. Foster, G. L.: Studies on Carbohydrate Metabolism, I, Some Comparisons of Blood Sugar Concentrations in Venous Blood and Finger Blood, J. Biol. Chem. **55**:291-301 (Feb.) 1923.

6. Foster (Footnote 5). Cori, C. F., and Cori, G. T.: Comparative Study of the Sugar Concentration in Arterial and Venous Blood During Insulin Action, Am. J. Phys. **71**:688 (Feb.) 1925.

PANCREATIC FUNCTION

I. THE QUANTITATIVE DETERMINATION OF PANCREATIC ENZYMES *

EDWARD HOLLANDER, M.D.
AND
JOSEPH M. MARCUS, M.D.
NEW YORK

Enzymes are substances produced by living cells which behave like catalysts in that they change the rate of chemical reactions without themselves undergoing any apparent physical or chemical change. Since enzymes have not been isolated in a pure state, our knowledge of their properties is limited to the study of the reactions that they accelerate or retard.

In quantitative tests of enzymatic activity, a fixed proportion must exist between the amount of enzyme that is present and the effect that is produced. It is therefore essential that the laws governing their velocity of reaction should be properly applied.

According to the law of mass action, the rate of reaction is proportional to the concentration of the reacting molecules. At any moment the velocity of reaction is proportional to the amount of substance that remains undecomposed. In a unimolecular reaction of an inorganic catalyst, such as the hydrolysis of cane sugar by hydrochloric acid,

$$\frac{dx}{dt}, \text{ or velocity of hydrolysis, } = KF(a-x) \qquad (1a)$$

a = the initial amount of substrate; x = the amount of substrate that has been hydrolyzed at the moment of observation; k = a constant expressing the ratio between the mass of remaining substrate and the velocity of hydrolysis, and F = the amount of catalyst present. Here the intensity of the reaction is in direct proportion to the concentration of the enzyme.

The relation between the mass of substrate hydrolyzed and the time of hydrolysis is found by integration to be $\log_e \frac{a}{a-x} = \mathrm{KFt}$ (1b). Such a relationship when plotted forms a logarithmic curve.

With enzymes the rate of reaction is not expressed in terms of the equation just given for inorganic catalysts. The velocity of reaction is dependent on many factors, the more important ones being (*a*) colloidal adsorption, (*b*) reversibility of reaction, and (*c*) the effect of the products of the reaction.

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* From the department of physiologic chemistry, Mt. Sinai Hospital.

COLLOIDAL ADSORPTION

In the hydrolysis of sucrose by an enzyme, such as invertase, when the proportion of sugar to invertase is high, the rate of hydrolysis is constant for a given amount of invertase. For varying amounts of invertase the velocity of hydrolysis is proportoinal to the concentration of the enzyme.

Thus, instead of a logarithmic equation, we have

$$\text{Velocity of hydrolysis} = KF \qquad (2a)$$

The relation between the quantity of substrate hydrolyzed (x) and the time of hydrolysis (t) must be $x = KFt$ (2b)

Amylopsin in its action on starch follows this law. Here the quantity of substrate changed in any interval of time is a constant amount. The curve of reaction instead of being a logarithmic one becomes a straight line. As the action proceeds, when the substrate is insufficient to bind all of the enzyme, the amount of the substrate hydrolyzed is directly proportional to its concentration, as the logarithmic law requires (Formula 1a and 1b).

This type of reaction can be explained only by the assumption that a compound of enzyme and substrate is formed which exists for an appreciable time (adsorption). All reactions brought about by enzymes occur in a heterogeneous system. The active mass of substrate is unknown. In the hydrolysis of sucrose by acids, the amount of acid that is bound by the sucrose at any instant is so small that it has never been quantitatively determined. In such a case the active mass of substrate is the amount of substrate that is undecomposed.

REVERSIBILITY OF REACTION

All reactions catalyzed by enzymes are reversible. The enzyme combines with the end products of the substrate and then it acts in opposition to its first combination (synthesis). In hydrolytic reactions the rate of hydrolysis is retarded as equilibrium is approached. The velocity of hydrolysis at any moment is the difference between the two opposite reactions of hydrolysis and synthesis; in final equilibrium no apparent change takes place, although in reality the condition is not one of rest but of balance between these two opposing processes each acting at a definite rate.

The rôle of reversibility of reaction is important in the study of the velocity of reactions with steapsin and trypsin. In the case of amylopsin the factor of reversibility is negligible since its hydrolytic reaction progresses almost to completion before any reverse action occurs.

In reversible reactions in which the enzyme is bound and inactivated by the products of hydrolysis, the velocity of reaction will be inversely proportional to the extent of the hydrolysis:

$$\frac{dx}{dt}, \text{ or velocity of hydrolysis,} = \frac{KF\ (a-x)}{x}$$

In the early stages of the reaction, when x is small, $a-x$ is nearly equal to a:

$$\frac{dx}{dt}, \text{ or velocity of hydrolysis,} = \frac{KFa}{x} \qquad (3a)$$

On integration,

$$X = \sqrt{2KFat} \qquad (3b)$$

which is the Schütz-Borissov law; namely, that the intensity of action is directly proportional to the square root of the concentration of the enzyme. This rule holds good, however, only during the early stages of the hydrolysis.

EFFECT OF THE PRODUCTS OF HYDROLYSIS

In digestion by trypsin or by steapsin, amino-acids or fatty acids are produced which cause a reduction in hydroxyl ion concentration. This has a destructive action on these enzymes as the optimum hydrogen ion concentration for their activity becomes changed. Hence, it is important in the study of the velocity of reaction of these enzymes to maintain the hydrogen ion concentration at about its optimal value throughout the period of observation.

It follows from the principles discussed that a general law cannot be stated for the relation between the velocity of a reaction and the concentration of an enzyme. In some cases, as in the hydrolysis of sucrose by invertase or in the hydrolysis of starch by amylase, the relation is a direct linear one. Generally, the proportion is expressed in some exponential form as in the square root law of Schütz-Borissov. However, this law is only of limited application during the early stages of certain reactions. The ratio is generally less than the square root.

The only universal rule that has been found to govern the action of the hydrolyzing enzymes is the "time law" of Hedin. The time required to obtain the same effect with varying amounts of an enzyme varies inversely with the concentration of the enzyme. In quantitative determinations of reversible reactions especially, it is more accurate to measure the time taken to effect a definite change than the amount of change in a definite time.

All clinical methods thus far proposed for the quantitative determination of pancreatic ferments have been based on the determination of the amount of enzymatic activity on various substrates at the end of a fixed interval of time (from one to twenty-four hours). As discussed above, only in the case of amylopsin is there a direct linear

proportionality between the amount of enzyme present and the amount of hydrolysis effected, when the substrate is in excess. (The velocity of reaction follows equation 2a and 2b.) In Table 1 is given the number of cubic centimeters of digestion mixture required to reduce 5 c.c. of Benedict's copper solution. To flasks containing 50 c.c. of 3 per cent. aqueous solution of soluble starch, 1 c.c., 0.5 c.c., 0.25 c.c. and 0.125 c.c. of the same specimen of duodenal contents were added. These were incubated for one-half hour at 37.5 degrees C.[1]

These results, within the limits of experimental error, follow the law of direct linear proportionality. Benedict's solution is reduced

TABLE 1.—*Cubic Centimeters of Starch Digestion Mixture Required to Reduce Five Cubic Centimeters of Benedict's Solution*

Amount of Duodenal Contents Used	Case 1	Case 2	Case 3	Case 4	Case 5
1 c.c.	0.82	0.85	1.30	1.20	1.30
0.5 c.c.	1.48	1.70	2.60	2.30	2.65
0.25 c.c.	2.56	3.00	5.30	4.40	5.65
0.125 c.c.	5.28	6.05	11.45	9.00	11.60

TABLE 2.—*Determination of Tryptic Activity (Method of Lueders, Bergeim and Rehfuss)*

Amount of Duodenal Contents Used	Amount of Tenth Normal Sodium Hydroxid Required to Bring Digestion Mixture to Neutral Point				
	Case 1	Case 2	Case 3	Case 4	Case 5
1 c.c.	6.1	6.5	6.0	7.2	3.7
0.5 c.c.	5.8	5.6	4.8	5.7	2.6
0.25 c.c.	3.9	3.3	3.7	4.3	1.3

TABLE 3.—*Determination of Lipolytic Activity (Method of Lueders, Bergeim and Rehfuss)*

Amount of Duodenal Contents Used	Amount of Tenth Normal Sodium Hydroxid Required to Bring Digestion Mixture to Neutral Point				
	Case 1	Case 2	Case 3	Case 4	Case 5
1 c.c.	9.0	9.3	1.9	5.0	5.5
0.5 c.c.	6.0	5.2	1.0	4.0	4.1
0.25 c.c.	4.3	4.1	0.7	3.2	2.6

only by the maltose formed. The fact that the hydrolysis of starch occurs in stages, with the formation of several dextrins as well as maltose, does not produce any appreciable error.

In the case of trypsin (Table 2) and of steapsin (Table 3) no simple proportionality was found between the amount of enzyme used and the amount of hydrolysis effected. This is in accord with the

1. To stop further activity of the amylopsin, a small amount of sodium carbonate should be added to each flask. In the reduction of Benedict's solution, the digestion mixture can be added rapidly till a milky precipitate appears. Further addition should be by drops with constant boiling for several seconds between each drop.

theoretical analysis of the behavior of these enzymes discussed above. The method used was that of Lueders, Bergeim and Rehfuss, which was considered the best clinical method recently proposed. The method recently advanced by McClure, in which buffers are used to maintain a constant hydrogen ion concentration throughout the reaction, was considered too complicated and time consuming to be of clinical value and was therefore not employed. In the method of Lueders, Bergeim and Rehfuss, lipase and steapsin act on a 20 per cent. emulsion of olive oil and on a 5 per cent. aqueous solution of gelatin, respectively, for one hour. The amount of fatty acids or amino-acids formed is determined by titration with tenth normal sodium hydroxid.

To obtain a simple and exact relationship in the quantitative determination of trypsin and steapsin we have applied the "time law" of Hedin in the following manner: Trypsin and steapsin are both active in a neutral or a slightly alkaline medium. Phenolphthalein begins to turn red at a p_H of 8.4. If 1 c.c. of tenth normal sodium hydroxid is then added to 25 c.c. of the substrate, a deep red results and the hydrogen ion concentration is not changed sufficiently to inhibit the enzyme. The time required for the hydrolytic activity (amino-acid or fatty acid formation) to neutralize this 1 c.c. of tenth normal sodium hydroxid and cause the disappearance of all pink in the substrate is in inverse ratio to the concentration of the enzyme.

With steapsin (Table 4) 10 per cent. olive oil emulsion [2] was used as substrate. To 200 c.c. of olive oil emulsion, 2 c.c. of 1 per cent. alcoholic solution of phenolphthalein is added by the addition of tenth normal sodium hydroxid the emulsion is brought to a permanent light pink. Twenty-five cubic centimeters of this neutralized oil is placed in each of eight 30 c.c. test tubes of uniform size. To each of four of these tubes 1 c.c. of tenth normal sodium hydroxid is added, which produces a deep red. The four remaining test tubes containing 25 c.c. of neutralized oil are used as controls. The tubes are placed in a water bath at 37.5 degrees C., and this temperature is maintained throughout the period of observation. To each tube and its control the same amount of duodenal contents is added from a 1 c.c. pipet graduated to 0.01 c.c. The pipet is inserted to the bottom of the tube and its contents are gently blown into the substrate of oil while the pipet is being withdrawn. In this way practically none of the ferment is lost. The test tube is then shaken a few times by hand. The time required for the disappearance of all pink by comparison with the control tube

2. To make olive oil emulsion (10 per cent.) 50 gm. of gum arabic should be placed in a mortar and 50 c.c. of pure olive oil added and rubbed into a smooth paste. Then 50 c.c. of distilled water should be added and constantly stirred until white emulsion is formed. More water, to a total volume of 500 c.c., should be gradually added. This should be kept in an icebox.

(neutralization of 1 c.c. of tenth normal sodium hydroxid by the fatty acids developed) is determined in seconds and minutes. When the pink is gone the tube is of a slightly lighter yellow than the control tube, owing to the extra 1 c.c. of tenth normal sodium hydroxid that was

TABLE 4.—*Time Required for Disappearance of All Pink (Neutralization of 1 C.c. of Tenth Normal Sodium Hydroxid by Fatty Acids)*

Amount of Duodenal Contents Used	Case 1	Case 2	Case 3	Case 4	Case 5
0.4 c.c.	½ min.	2 min.	¾ min.	½ min.	50 sec.
0.2 c.c.	1¼ min.	4 min.	1½ min.	1¼ min.	2 min.
0.1 c.c.	2½ min.	9 min.	3 min.	2¾ min.	5 min.
0.05 c.c.	6 min.	17 min.	6 min.	5½ min.	10½ min.

TABLE 5.—*Time Required for Disappearance of All Pink (Neutralization of 1 C.c. of Tenth Normal Sodium Hydroxid by Amino-Acids)*

Amount of Duodenal Contents Used	Case 1	Case 2	Case 3	Case 4	Case 5
1 c.c.	3 min.	1½ min.	10 min.	7 min.	3 min.
0.5 c.c.	6 min.	3 min.	20 min.	13 min.	6 min.
0.25 c.c.	12 min.	7 min.	41 min.	30 min.	13 min.
0.125 c.c.	24 min.	14 min.	Not determined	57 min.	25 min.

Protocol of Case 1

Amount of Duodenal Contents Used	Minutes After Addition of Enzyme to Substrate	Remarks
1 c.c. added when second hand of watch was at 60	1	Red
	1	Pink
	1	All pink gone
	1	All pink gone (no further change)
Conclusion: Reaction complete in three minutes		
0.5 c.c. added when second hand of watch was at 00	3	Red
	1	Pink
	1	Light pink
	1	All pink gone
	1	All pink gone (no further change)
Conclusion: Reaction complete in six minutes		
0.25 c.c. added when second hand of watch was at 00	6	Red
	2	Pink
	3	Light pink
	1	All pink gone
	1	All pink gone (no further change)
	1	All pink gone (no further change)
Conclusion: Reaction complete in twelve minutes		
0.125 c.c. added when second hand of watch was at 60	7	Red
	8	Pink
	4	Light pink
	2	Faint pink
	1	Faint pink by comparison with control
	1	Faint pink by comparison with control
	1	All pink gone
	1	No further change
	1	No further change
Conclusion: Reaction complete in 24 minutes		

added. In the tubes with the smaller quantities of enzymes the pink changes slowly. In these, the reaction is watched for a few minutes after all the pink has disappeared to make certain that no further change occurs (Case 1, Table 5).

With trypsin (Table 5) 5 per cent. gelatin in an emulsion of mineral oil [3] was used as substrate. The same technic was employed as described for steapsin, except that larger amounts of duodenal contents were used.

Within the limits of experimental error, these tables show that the time required to neutralize 1 c.c. of tenth normal sodium hydroxid is in inverse ratio to the amount of ferment present. The clinical application of these methods will be the subject of a future report.

SUMMARY

1. Present clinical methods for the quantitative determination of pancreatic ferments are based on the determination of the amount of enzymatic activity obtained at the end of a fixed interval of time (from one to twenty-four hours).

2. However, in reversible hydrolytic reactions no definite proportionality exists between the velocity of a reaction and the concentration of an enzyme. Only in the case of amylopsin, in which the factor of reversibility is negligible, does a direct linear proportionality exist between the amount of enzyme present and the amount of hydrolysis effected.

3. In reactions with trypsin and steapsin a linear proportionality can be obtained by the application of the "time law" of Hedin: The time required to obtain the same effect with varying amounts of an enzyme varies inversely with the concentration of the enzyme.

4. We have applied the "time law" of Hedin in a simple manner that can be employed in quantitative tests for these ferments.

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3. Gelatine emulsion (5 per cent.) is milky white and forms an excellent contrast medium for the pink of the phenolphthalein. Aqueous gelatin solution is light brown and does not contrast well with a pink. To make the emulsion 25 gm. of highest grade gelatin should be added to 300 c.c. of distilled water and gradually heated to 60 degrees C. till the gelatin is completely dissolved. Fifty grams of best grade (whitest grade) gum arabic and 50 c.c. of liquid petrolatum are placed in a mortar and rubbed into a smooth paste; then 50 c.c. of distilled water is gradually added with continual stirring. When a homogeneous paste is formed the heated gelatin solution should be added gradually and stirred continually. A milky white emulsion is then formed. Hot distilled water should be added slowly till the total volume is 500 c.c. This should be kept in the icebox in 50 c.c. flasks. When needed these are liquefied by gentle heat in a water bath.

Book Review

CLINICAL FEATURES OF HEART DISEASE. AN INTERPRETATION OF THE MECHANICS OF DIAGNOSIS FOR PRACTITIONERS. By LEROY CRUMMER, M.D., Professor of Medicine, University of Nebraska. With an introduction by EMANUEL LIBMAN, M.D., Physician to Mount Sinai Hospital; Professor of Clinical Medicine, Columbia University, New York. New York: Paul B. Hoeber, 1925.

Few books have been written in recent years on disease of the heart in which considerable space has not been devoted to the newer methods in cardiac diagnosis. Not infrequently there has been a tendency to overestimate the value of the instruments of precision. This book by Crummer, with introduction by Libman, is an exception to the general tendency in that the mechanical aids employed in cardiac diagnosis are considered in a short chapter of fifteen pages.

Emphasis is placed on the value of the simple methods of physical examination and careful clinical observations and at this time this fully justifies a work of this type. The book is apparently not intended to make an extensive survey of the entire field. A creditable balance is, however, maintained and several suggestions are made which should stimulate further careful clinical observation. The book is easily read. It will be a helpful guide to the practitioner and of interest and value to those particularly interested in cardiac disease.